

1 First comparative approach to touchscreen-based visual object-location paired-
2 associates learning in humans and a non-human primate.

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10

11 **Abstract**

12

13 A recent study suggests that the touchscreen-based dPAL task on visual object-location paired-
14 associates learning (PAL) allows effective translation from animal models to humans. Here, we
15 adapted the task to a non-human primate (NHP), the grey mouse lemur, and provide first evidence for
16 the successful comparative application of the task to humans and NHPs.

17

18 Young human adults reach the learning criterion after considerably less sessions (one order of
19 magnitude) than young, adult NHPs, which is likely due to faster and voluntary rejection of ineffective
20 learning strategies in humans and almost immediate rule generalization. At criterion, however, all
21 human subjects solved the task by either applying a visuo-spatial rule or, more rarely, by memorizing
22 all possible stimulus combinations and responding correctly based on global visual information. An
23 error-profile analysis in humans and NHPs suggests that successful learning in NHPs is comparably
24 based either on the formation of visuo-spatial associative links or on more reflexive, visually-guided
25 stimulus-response learning. The classification in the NHPs is further supported by an analysis of the
26 individual response latencies, which are considerably higher in NHPs classified as spatial learners.

27

28 Our results, therefore, support the high translational potential of the standardized, touchscreen-based
29 dPAL task by providing first empirical and comparable evidence for two different cognitive processes
30 underlying visual object-location paired-associates learning in primates.

31

32 **Keywords**

33 Paired-associates learning; Comparative cognition; Primates; Intelligence; Evolution

34

35 **Introduction**

36 One of the major challenges in evolutionary cognitive and biomedical research is the development of
37 standardized cognitive testing procedures that allow for the comparative assessment of cognitive
38 functions and malfunctions in specific domains. An increasingly popular approach to this problem is
39 the adaptation of non-verbal, computerized tasks initially developed for human diagnostics to animals
40 (e.g. (Bussey et al., 2012; Horner et al., 2013; Oomen et al., 2013)). While this strategy often led to
41 valuable results in the past, the process of adaptation inherently involves the risks that task validity
42 gets lost and that positive results in animal studies are potentially over-interpreted in an
43 anthropomorphic sense. The former risk usually increases the longer the phylogenetic distance from
44 humans - from great apes over smaller non-human primates (NHP) to rodents -, i.e. with an increasing
45 need of protocol reductions due to both cognitive and physiological/motoric constraints of the chosen
46 model species. Therefore, the opposite approach, to use standardized tasks from animal cognitive
47 research to assess conserved cognitive functions in humans, was recently proposed (Nithianantharajah
48 et al., 2015).

49 An animal protocol that likely fits this purpose is the dPAL task (the “d” in dPAL stands for
50 “different”) on visual object-location paired-associates learning. It was developed for rodent testing
51 (Talpos, Winters, Dias, Saksida, & Bussey, 2009) and requires the subjects to procedurally learn to
52 discriminate three different visual items (black-and-white shapes) and to associate each of them with
53 one out of three possible locations on a touchscreen. At a given trial of the task, two of the three items
54 are presented simultaneously, one as a rewarded item-place match and the second, *different* one as an
55 unrewarded item-place mismatch. The dPAL task was found to be sensitive to pharmacological
56 manipulations and targeted lesioning in rodents and involves hippocampus-based spatial processing
57 and/or striatal stimulus-response learning (e.g. (Delotterie et al., 2015; C. H. Kim, Heath, Kent,
58 Bussey, & Saksida, 2015; M. Kim, Kwak, Yu, & Kaang, 2016; Talpos et al., 2009)). In that respect, it
59 differs from the closely related sPAL task (item-place match and item-place mismatch of a given trial
60 are the “*same*” item presented as duplicate), in which sensitivity to pharmacological manipulations of
61 the hippocampus seems to be missing (Talpos et al., 2009). A suggested reason for this insensitivity of
62 the sPAL task to hippocampal manipulations is that it favours the utilization of a hippocampus-

63 independent conditional rule (Talpos et al., 2009). Further, a distinction has to be made between the
64 dPAL task and the Human CANTAB PAL protocol: The latter requires the trial-unique formation and
65 delayed retrieval of visuo-spatial paired associates and has very recently been validated against
66 established neuropsychological measures of episodic memory (Lenehan, Summers, Saunders,
67 Summers, & Vickers, 2016). This means that Human CANTAB PAL assesses a different construct in
68 which both memory encoding and retrieval depend on medial-temporal structures (hippocampus
69 proper and parahippocampal gyrus, respectively (de Rover et al., 2011); compare (Takahashi, Ohki, &
70 Miyashita, 2002)). In dPAL, learning occurs procedurally, i.e. it is not a model for episodic or
71 episodic-like memory in humans and animals, respectively. However, applying the dPAL task to mice
72 and humans, it could be demonstrated that a human sample with disease-related Dlg2 deletions shows
73 deficits in visuo-spatial paired associates learning parallel to those found in a sample of Dlg2 knockout
74 mice (Nithianantharajah et al., 2015). Based on this finding, it was postulated that animal protocols,
75 such as the dPAL task, could effectively be used to bridge the translational gap from animal models to
76 humans, by assessing cognitive mechanisms that presumably are conserved across species
77 (Nithianantharajah et al., 2015).

78 The first aim of our study was to train the grey mouse lemur (*Microcebus murinus*) in the
79 highly standardized dPAL protocol to provide first comparative performance data from a NHP. Mouse
80 lemurs are particularly suited for this purpose, as they are currently discussed as a natural, chronic
81 NHP model of human brain-aging and Alzheimer's disease (AD) that could be used to complement
82 the rodent models that are dominating the field (Joly, Ammersdorfer, Schmidtke, & Zimmermann,
83 2014; Schopf et al., 2014; Verdier et al., 2015; Verdier & Mestre-Francés, 2016): Mouse lemurs show
84 age-related loss of functionality in motoric, sensory, and cognitive domains that is similar to the
85 effects of senescence known from humans. In addition, some aged mouse lemurs *naturally* develop
86 neuropathological features of an AD-like neurodegenerative disease, such as amyloid plaques, tau
87 aggregation, and cerebral atrophy (for a concise overview see (Verdier & Mestre-Francés, 2016)).
88 Thus, different from transgenic rodent models, mouse lemurs allow for research on disease
89 development and, with maximum ages of up to 14 years in our colony, for longitudinal studies on
90 long-term disease progression. Despite their potential as a natural model, a full mouse lemur genome

91 reference has recently been published (Mmur_3.0: GenBank assembly accession: GCA_000165445.3)
92 and strategies for the establishment of a mouse lemur knockout library through a reverse-genetic
93 approach are currently discussed (Ezran et al., 2017). Standardized, touchscreen-based tools for the
94 assessment of appetitive conditioning learning and cognitive flexibility have recently been adapted to
95 this species (Joly et al., 2014). A comparable protocol for the assessment of hippocampal integrity in
96 mouse lemurs is currently missing, but urgently needed, as the hippocampal formation is among the
97 brain areas that are the first to be affected by Alzheimer's disease (e.g. (Arnold, Hyman, Flory,
98 Damasio, & Van Hoesen, 1991; Jack et al., 2000)). Apart from this biomedical aspect, mouse lemurs
99 belong to a group of nocturnal primates that are often considered to represent an ancestral primate
100 condition (Martin, 1990). Standardized, visuo-spatial PAL data from mouse lemurs would, thus,
101 provide novel insights into the evolution of intelligence for both biomedicine and evolutionary
102 anthropology. The second aim of the study was to additionally test a set of human subjects in dPAL
103 for comparative reasons and to link results to those of verbal post-acquisition interviews to determine
104 learning strategies in humans. Such data can help to identify comparable cognitive processes in
105 humans and NHPs to further bridge the translational divide.

106

107 **Materials and Methods**

108 *Research ethics.* Animal testing was in accordance with the NRC Guide for the Care and Use of
109 Laboratory Animals, the European Directive 2010/63/EU, and the German Animal Welfare Act. It was
110 approved by the Animal Welfare Committee of the University of Veterinary Medicine and approved
111 and licensed by the Animal Welfare Committee of the LAVES (ref. 33.12-42502-04-14/1454,
112 04/28/2014). All human subjects gave written informed consent to participating in the study and to the
113 publication of their anonymized data. The used methods were in accordance with the current ethical
114 guidelines of the German Psychological Society (DGP) and the American Psychological Association
115 (APA) and approved by the Ethics Committee of the Hannover Medical School (ref. 2833-2015).

116

117 *Subjects.* We trained a total of twelve adult individuals of the grey mouse lemur (*M. murinus*;
118 $N_{\text{female}}=8$; $N_{\text{male}}=4$; age range: 2-8 years) in the touchscreen-based dPAL protocol. Mouse lemurs were

119 born and kept at the breeding colony of the Institute of Zoology (University of Veterinary Medicine,
120 Hannover; Landeshauptstadt Hannover: ref. 42500/1H, 01/15/2014; for details on animal housing see
121 (Joly et al., 2014)). As intact vision plays a vital role in touchscreen-based cognitive testing, all NHPs
122 considered for the study had been checked for ocular pathologies by a veterinarian ophthalmologist
123 prior to testing (for methods compare (Dubicanac et al., 2016; Dubicanac, Radespiel, & Zimmermann,
124 2017; Dubicanac, Strueve, et al., 2017)). Only animals without any signs for impaired vision (e.g.
125 prolonged pupillary reflex, corneal anomalies, uveitis, and advanced cataracts) were used as subjects.
126 Furthermore, we tested twelve male, human adults (age range: 19-34 years) in the touchscreen-based
127 dPAL protocol. Human subjects were recruited on the campus of the University of Veterinary
128 Medicine. They were *naïve* as to the nature of the task.

129

130 *Setup, stimuli, and general testing procedure.* NHPs were tested on a daily basis with one session of
131 36 regular trials per animal and day. Testing took place during the first two hours of the animals'
132 activity periods and in a room separate from the housing rooms using a customized version of the
133 Bussey-Saksida Touchscreen Chamber (Model 80604, Campden Instruments LTD.; Fig. 1A) and a
134 self-coded dPAL protocol running on ABET-II (Model 89505, Lafayette Instrument). The chamber
135 had a symmetrically trapezoidal floor. The touchscreen was positioned at the long base (245 mm; front
136 end) of the isosceles trapezoid, whereas a reward tray (**RT**, Fig. 1A), through which liquid rewards
137 (apple juice) could be delivered, was positioned at the short base (130 mm; back end). The base-to-
138 base distance was 330 mm and the volume accessible by the NHPs had a height of 100 mm. The
139 touchscreen itself constituted the whole front wall of the chamber, but was covered by a black Perspex
140 mask with three response windows (**1-3**, Fig. 1A), through which the NHPs had access to the screen
141 and behind which the training items were presented (Fig. 1A). The response windows were square-
142 shaped (45 x 45 mm) and separated from the adjacent window(s) by a distance of 20 mm. In general,
143 only pictorial black-and-white items were used for training. For the actual dPAL, we chose the set of
144 stimuli initially introduced by Talpos and colleagues (flower, airplane, and spider; Fig. 1B; (Talpos et
145 al., 2009)) to allow highest possible comparability with preceding studies (e.g. (Bartko, Vendrell,
146 Saksida, & Bussey, 2011; Nithianantharajah et al., 2015; Talpos et al., 2009)).

147 Humans were tested on a single day per subject and in several consecutive sessions with 36
148 regular trials per session. To keep comparability between species as high as possible, human subjects
149 made their responses to a touchscreen from a disassembled Bussey-Saksida Touchscreen Chamber and
150 were trained in a highly similar dPAL protocol (for minor differences see below). Both NHPs and
151 humans were tested in the dark with the touchscreen being the only source of visible illumination.
152 During the tests, the experimenter monitored the subjects' performance from an adjacent room.
153
154 *dPAL in the NHP (M. murinus)*. Before the animals entered the dPAL task, they had to proceed
155 through a 5-step autoshaping procedure in which they had learned to interact with the touchscreen
156 chamber, i.e. to respond (by nose-poke or touch) to pictorial stimuli pseudo-randomly presented at one
157 out of three possible positions on the touchscreen (**1-3**, Fig. 1A; for details of the autoshaping
158 procedure compare (Joly et al., 2014)). In the dPAL task, animals had to learn to visually discriminate
159 three pictorial stimuli (flower, airplane, and spider; Fig. 1B) and to associate each of them with a
160 rewarded location on the touchscreen (see Video S1 for an example of a NHP performing the task).
161 The dPAL stimuli were new to all subjects. The rewarded location for each stimulus was kept constant
162 across trials and sessions (flower = "left"; airplane = "centre"; spider = "right"). At a given trial (for a
163 flowchart overview see Fig. S1), two of the three stimuli were presented simultaneously, one at its
164 rewarded location (**S⁺**), the other one at an "incorrect", unrewarded location (**S⁻**). The third response
165 window was left blank (**S[?]**, Fig. 1B). A response to the **S⁺** led to a reward (15 µl apple juice). Reward
166 collection triggered a 5 s inter-trial-interval (ITI), after which the next regular trial (new stimulus
167 combination) could be initiated by revisiting the reward tray (**RT**, Fig. 1A). A response to one of the
168 incorrect response windows (**S⁻**) was signalled by a brief pure tone (2 kHz, 0.5 s) followed by a 5 s
169 time-out and a 5 s ITI after which a correction trial (CT) could be initiated. During correction trials,
170 the stimulus combination to which the animal previously had responded incorrectly was presented
171 again and under the same conditions as a regular trial until the subject eventually responded to the **S⁺**.
172 Within a complete session of 36 regular trials, the six possible stimulus combinations (**SC₁-SC₆**,
173 Fig. 1B) were presented in a pseudo-randomized, balanced design. Animals were trained in the dPAL
174 protocol until they reached a performance of 80% correct choices (correction trials excluded) in two

175 consecutive, complete sessions. A session ended after 36 completed regular (non-correction) trials or a
176 maximum duration of one hour.

177

178 *dPAL in humans*. For human testing, the 5-step autoshaping was replaced by a short (10 trials) test
179 session, in which the subjects were allowed to freely interact with the touchscreen. All subjects
180 intuitively responded to the pictorial items presented pseudo-randomly at one of the three locations on
181 the touchscreen and proceeded quickly through the test session. The task was slightly modified, as
182 correct decisions were not physically rewarded, but signalled by a green checkmark presented at the
183 center of the touchscreen (at a position above the response windows used for stimulus presentation). A
184 red “x” was used to indicate incorrect responses to the subject. To initiate a new trial after the ITI had
185 passed, subjects had to press a “next” symbol at the same position. All other protocol parameters
186 (pictorial stimuli, sound of the reward pump, 2 kHz pure tone, ITI, time-out, number of trials/session,
187 etc.) were exactly as in the NHP version. Between sessions, subjects had free access to beverages
188 (water or caffeine-free lemonades) and sweets as compensation for their effort. After the learning
189 criterion (80% correct choices in two consecutive, complete sessions) had been reached by a given
190 participant, he was asked (I) for the rule that he believed was underlying the task and (II) whether he
191 had changed his strategy during dPAL.

192

193 *Statistics*. All statistical analyses were conducted with R (R 3.2.3, 2015, The R Foundation for
194 Statistical Computing). For descriptive statistics in Fig. 3B, mean and standard error of mean (\pm SEM)
195 are presented, to allow direct comparison with published data from the rodent literature. To test
196 individual error profiles for deviations from chance in the NHPs, we used χ^2 -based Goodness of Fit
197 statistics with Bonferroni correction for multiple testing. Only the last third of individual errors to
198 criterion was analysed to minimize the noise in the data caused by initial trial and error learning and/or
199 strategy switching. As the number of errors to criterion generally was high in the NHPs and even small
200 deviations from chance became significant as a result of the sample size, we additionally used
201 Cramer’s V (ϕ_c ; ‘lsr’ package in R) as an estimate of effect size. In humans, the number of errors to
202 criterion generally was too small to use comparable inferential statistics. Median response and reward

203 latencies were compared between NHPs using asymptotic Wilcoxon signed rank statistics. The
204 belonging effect sizes (r) were calculated from the Wilcoxon statistics as $r = z/\sqrt{N}$. Confidence
205 intervals for individual medians are presented as 95% bootstrap confidence intervals based on 10000
206 bootstrap samples each. A possible correlation between the number of errors/correction trials and the
207 number of self-reported assumed rules in human subjects was investigated using Spearman statistics.

208

209 **Results**

210 *dPAL performance in the NHP (M. murinus)*. Based on their global performance (learning curves), the
211 NHPs could be divided into three groups of individuals: (i) Animals belonging to the first group were
212 excluded from the study after a minimum of 50 sessions, if they regularly failed to complete sessions
213 of the dPAL task within the one-hour time limit (completion rates <25%; N=4; F₅-F₆, M₂-M₃; Fig. S2).
214 This decision was made, since the learning criterion in dPAL requires the subjects to achieve a
215 performance of at least 80% correct choices in two consecutive, *complete* sessions. In NHPs that
216 regularly fail to finish the sessions within the time limit, this criterion cannot be applied, as it either is
217 never reached or likely detects successful learning “too late”. The inclusion of incomplete sessions
218 was not an option: Performance measurements in these sessions often are biased towards low
219 percentages, as subjects usually stop responding after incorrect trials. Also, in incomplete sessions
220 with very low numbers of trials, extreme values of 0% or 100% regularly occur (compare Fig. S2), i.e.
221 a criterion including incomplete sessions can easily be reached without actual learning. (ii) Animals of
222 the second group eventually started to complete the dPAL sessions, but did not show any notable
223 increase in task performance after a minimum of 120 sessions (≥ 4 months of daily training), i.e.
224 performance fluctuated around chance level throughout the training (N=3; F₇-F₈, M₄; Fig. S3). (iii)
225 Finally, animals of the third group eventually completed the dPAL sessions and reached the *a priori*
226 learning criterion of 80% correct choices in two consecutive, complete sessions (N=5; one male: M₁;
227 four females: F₁-F₄). F₁ reached this criterion after 2158 (+1454 correction trials = CT; 66 sessions;
228 approx. 2 months) regular trials. F₂ and M₁ needed 2697 (+2011 CT; 76 sessions; approx. 2.5 months)
229 and 2940 (+1642 CT; 85 sessions; approx. 3 months) regular trials, respectively (Fig. 2A, 3A). The
230 two successful, aged adults (>7 years) reached the criterion after 5022 (F₄; +3398 CT; 150 sessions;

231 approx. 5 months) and 10207 (F₃; +6749 CT; 285 sessions; approx. 9.5 months) regular trials (Fig. 2B,
232 3A). Different from the dropouts, all successful NHPs showed a high tendency to complete the
233 training sessions (completion rates ranging from 83.3 to 98.6%) and a continuous performance
234 increase throughout the training (Fig. 2).

235 In order to learn more about the strategies used for task completion in the successful NHPs,
236 we analysed the terminal errors (last third of the errors made; Tab. 1) of these five individuals. Error
237 profiles were analysed separately for stimulus combination pairs with identical items (SC₁/SC₆,
238 SC₂/SC₄, SC₃/SC₅; Fig. 4A) and stimulus combination pairs with identical S⁺ (SC₁/SC₂, SC₃/SC₄,
239 SC₅/SC₆; Fig. 4B). For the first case (stimulus combination pairs with identical items), the error
240 distribution differed highly significantly from chance (33.3%; χ^2 -test; Bonferroni corrected $p < 0.01$) in
241 M₁, F₃, and F₄ (Fig. 4A), but only in M₁ the belonging effect was of a medium size (Cramer's V
242 = $\varphi_c = 0.229$) with an overrepresentation of terminal errors in SC₁/SC₆. All other effect sizes were small
243 or neglectable ($\varphi_c \leq 0.095$). For the second case (stimulus combination pairs with identical S⁺), the
244 error distribution differed significantly from chance (33.3%; χ^2 -test; Bonferroni corrected $p < 0.001$;
245 $\varphi_c \geq 0.19$) in all subjects, with an overrepresentation of terminal errors in SC₃/SC₄ and medium effect
246 sizes ($\varphi_c = 0.19$ to 0.25) in F₁-F₄ (Fig. 4B). This difference in error profiles between the male NHP and
247 the females was accompanied by differences in the individual, median response latencies (Tab. 2). M₁
248 showed a very low (1.78 s) median response latency (time interval between the onset of a given
249 stimulus presentation and the touchscreen response by the animal) as compared to the other four
250 individuals (F₁-F₄), for which the median response latencies were 1.5 to 2.5 times higher (2.59-4.37 s;
251 compare Tab. 2 and Fig. S4A for a density histogram of the individual response latencies). The
252 belonging median reward latencies, however, were low in all animals (M₁: 1.12 s; F₁-F₄: 0.92-1.32 s)
253 and individual differences were much smaller than those observed for the response latencies (compare
254 Tab. 2 and Fig. S4B for a density histogram of the individual reward latencies).

255
256 *dPAL performance in humans.* To investigate the range of possible strategies that can be used to reach
257 the task criterion in dPAL, we tested a set of twelve human subjects that were later (during post-
258 acquisition interviews) asked to verbally report the strategies they used. All humans reached the

259 criterion for task completion within 2-4 sessions, i.e. considerably faster than the other non-human
260 mammals that have been tested in dPAL (i.e. rats (Talpos et al., 2009), mice (Bartko et al., 2011), and
261 mouse lemurs; compare below), so far. Nevertheless, we could observe high inter-individual
262 differences in the number of errors the human subjects made until criterion (correction trials in
263 Fig. S5). These inter-individual differences were linked to differences in the number of possible rules
264 the subjects rejected before they eventually found the correct one ($r_{\text{Spearman}}=0.87$, $N=12$, $p=0.0002$;
265 compare Tab. S1). When asked for the suspected rule that underlies the task during individual post-
266 acquisition interviews, 10 out of 12 subjects (S₁-S₇, S₁₀-S₁₂; Fig. 3A) correctly reported the object-
267 location paired-associates rule underlying the paradigm and confirmed it as the one they consequently
268 employed to reach criterion (Tab. S1). The two remaining subjects (S₈-S₉) reported to have memorized
269 all possible stimulus combinations (SC₁-SC₆) and the belonging correct responses to solve the task,
270 without recognizing a general rule (Tab. S1). Using this strategy, the latter two subjects belonged to
271 the least effective human participants (Fig. 3A; compare Fig. S5 for the non-logarithmic graph). This
272 allowed an analysis of their error profiles comparable to the NHPs, in which both subjects, just like
273 NHP M₁, showed a clear bias for errors in trials with either SC₁ and/or SC₆ being presented (Fig. 4A).

274

275 *Comparative data on dPAL learning dynamics in non-human mammals.* For the sake of completeness,
276 we compared the grouped learning curves of the successful, young NHPs (≤ 4 years) with the grouped
277 learning curves reported for young rats (Talpos et al., 2009) and young mice (Bartko et al., 2011). The
278 data reveals that learning performance in the NHP lies within the same range as learning performance
279 in rodents (Fig. 3B). This comparison, however, is based on grouped learning dynamics alone and
280 does not allow for a comparison of individual learning strategies involved in dPAL between the
281 species. Comparative data on the error profiles in mice and rats, unfortunately, had not been available.

282

283 **Discussion**

284 The here-presented results are the first demonstration of a successful comparative application of the
285 dPAL protocol in a non-human primate and humans. The study further provides a first analysis of
286 possible solving strategies in humans and shows that humans can reach the task criterion using two

287 different strategies. They can solve the task either by a memorizing strategy, using the gross visual
288 appearance of the presented stimulus combinations to learn the belonging correct responses, or by
289 applying a spatial rule. As intended by the developers of the task (Talpos et al., 2009), the latter
290 strategy includes the formation of visual object-location paired-associates (i.e. the mapping of
291 different items onto absolute spatial positions) and was the one predominantly used in the human
292 subjects. The finding of two distinct error profiles and response dynamics in the successful NHPs
293 suggests a highly similar dissociation of two different solving strategies in mouse lemurs with a
294 dominance of the spatial strategy, as we will discuss in the following paragraphs. We will start,
295 however, with a discussion of the unsuccessful NHPs and suggestions on how their numbers can
296 potentially be reduced in future studies on dPAL.

297

298 *dPAL in the unsuccessful NHPs.* Of the 12 tested NHPs, only 5 could successfully be trained to
299 criterion. One possible interpretation of these results is that the behaviour shown by the successful
300 animals is atypical for mouse lemurs. Based on the observations we made during the training and our
301 experience with touchscreen-based testing in mouse lemurs from previous studies (e.g. (Joly et al.,
302 2014)), however, we think that this is unlikely. Instead, we suggest that the observed "failure" of some
303 of the NHPs was due to protocol features that can readily be modified to potentially increase the
304 number of successful learners without negative effects on construct validity: (i) For the unsuccessful
305 NHPs that were excluded from the study after at least 50 sessions (N=4), as they regularly failed to
306 complete sessions within the one-hour time limit, the main problem seemed to be a motivational one.
307 We assume that the observed behaviour resulted most likely from the rule change between the last
308 autoshaping sessions (every response to a pictorial stimulus is rewarded), which all subjects had
309 regularly finished within the time-limit, and the actual dPAL task (only the item-place match is
310 rewarded, whereas the item-place mismatch is not). This rule change inevitably entailed a sudden,
311 considerable increase in the reward-work requirement that may have exceeded the motivational level
312 of some of the subjects. As stated above, this does not mean that these subjects were unable to learn
313 the task *per se*. It rather means that they would have needed a (much) higher number of absolute
314 training days to improve dPAL performance and, more critically, that they could not reach the pre-

315 defined task criterion, which required them to complete the sessions. To possibly circumvent these
316 problems in the future, we suggest two alternative modifications to the protocol. In order to increase
317 the motivational level of the subjects at the time of the rule change to counteract the increase in
318 reward-work requirement, one could slightly reduce the subjects' food/caloric intake during the days
319 of the very first dPAL sessions. This modification would be easy to implement, but has ethical
320 implications that would have to be taken into consideration. It, therefore, could only be applied in a
321 very limited range. A second, less critical approach in terms of ethical considerations would be the
322 realization of a home-cage based training procedure with free access to the setup and a rolling criterion
323 instead of the session-based training. While being a more elaborate solution and probably more
324 difficult to implement, such a procedure would prevent that subjects have to be removed due to
325 unfinished sessions and likely reduce the absolute number of training days by increasing the amount of
326 daily training. (ii) Of the remaining three dropouts, which were removed after at least 120 sessions (all
327 successful young NHPs reached the training criterion between the 66th and 85th session), as their
328 performance still fluctuated around chance level (50%), two had a clear stimulus preference, which
329 they failed to overcome despite the correction procedure. The reason for failure in the third animal is
330 unclear. We think that the number of dropouts of this type can effectively be reduced by changing the
331 set of stimuli that constitute the different stimulus combinations from pictorial items to more
332 featureless items. While the "flower-plane-spider" set of stimuli was the one routinely used in dPAL at
333 the time the here-reported experiments were conducted (e.g. (Bartko et al., 2011; M. Kim et al., 2016;
334 Talpos et al., 2009); Fig. 1B) and was chosen to guarantee a maximum degree of comparability, we
335 would consider using the set of line stimuli introduced by Kim and colleagues (C. H. Kim et al., 2015)
336 to minimize the negative effect of stimulus preferences on learning in future studies.

337

338 *dPAL in the successful NHPs.* Within the successful NHPs, we could distinguish two different error
339 profiles and response dynamics: One NHP (M_1) showed an error-profile with an overrepresentation of
340 errors in SC_1/SC_6 trials among the last third of individual errors made (Fig. 4A). This pattern suggests
341 a stimulus-response strategy in M_1 , as such a strategy would, just like in the humans who memorized
342 all possible stimulus combinations (compare below), either be based on differentiating the gross visual

343 appearance of the stimulus combinations or on recognizing the sequence (e.g. from left to right) of
344 individual items. Both the gross visual appearance and the sequence of individual items are highly
345 similar in SC₁/SC₆ (Fig. 4A), so that this stimulus combination pair can be expected to be the most
346 difficult to learn for individuals using a stimulus-response strategy. The remaining NHPs (F₁-F₄), on
347 the other hand, showed a bias towards the pair of stimulus combinations in which the S⁺ was presented
348 in the centre position (SC₃/SC₄; Fig. 4B). This pattern is indicative for a spatial strategy in F₁-F₄, as
349 SC₃/SC₄ is the most challenging stimulus combination pair in terms of spatial processing: Firstly, the
350 rewarded S⁺ is in the centre position. The respectively corresponding item-place mismatches (S⁻),
351 therefore, change position from left (in SC₃) to right (in SC₄, Fig. 4B). In all other stimulus
352 combination pairs with identical S⁺ (SC₁/SC₂, SC₅/SC₆), the corresponding item-place mismatches are
353 always on the same side (Fig. 4B). Secondly, in both SC₃ and SC₄ the corresponding item-place
354 mismatches are directly adjacent to the S⁺ (Fig. 4B), i.e. this stimulus combination pair has an
355 increased difficulty in terms of location discrimination as compared to SC₁/SC₂ and SC₅/SC₆ with a
356 larger spatial distance between S⁺ and S⁻ in one stimulus combination per pair (Fig. 4B). In line with
357 this, those of our NHPs that were classified as spatial learners (F₁-F₄) showed an increased (factor: 1.5
358 to 2.3) mean error frequency in stimulus combinations with narrow spatial distance between S⁺ and
359 item-place mismatch (SC₁, SC₃, SC₄, SC₆) as compared to the mean error frequency in stimulus
360 combinations with large spatial distance between S⁺ and item-place mismatch (SC₂, SC₅). This was not
361 the case in the NHP that was classified as a non-spatial learner (M₁; factor: 0.9; compare Tab. 1). In
362 further support of the classification of M₁ as a non-spatial learner and F₁-F₄ as spatial learners, M₁
363 showed a very low median response latency as compared to F₁-F₄ (Tab. 2 and Fig. S4A), while the
364 median reward latency of M₁ was well within the range of the other subjects (Tab. 2 and Fig. S4B).
365 This means that the special position of M₁ in terms of response latencies was not due to a motoric or
366 motivational advantage of M₁, but that the short response latencies in M₁ are likely to mirror fast,
367 reflexive decisions for a given response window based on visual stimulus appearance alone, whereas
368 the significantly longer response latencies in the remaining individuals are likely to be caused by
369 longer lasting decision-making processes that take both stimulus identity and position into account.

370 It is intriguing, that the NHP classified as non-spatial learner was the male individual among
371 those subjects who reached criterion, whereas the spatial learners all were females. It is well described
372 in the literature on both humans and rodents that internal levels of gonadal steroids can modulate
373 learning strategies. Female rats that were tested in a continuously rewarded spontaneous alternation task
374 in a Y-maze and a food finding task in a T-maze, for example, showed a bias towards spatial strategies
375 at pro-oestrous (high levels of ovarian steroids), whereas female rats at oestrous preferentially used
376 response strategies in the same tasks (Korol, Malin, Borden, Busby, & Couper-Leo, 2004).
377 Comparably, in humans, women tested in a virtual navigation task at high progesterone levels during
378 the mid/late luteal phase also showed a bias towards spatial strategies (Hussain, Hanafi, Konishi,
379 Brake, & Bohbot, 2016). While it is unclear, whether the distribution of spatial and non-spatial
380 learners between the sexes we observed is pure coincidence, we can likely exclude the possibility that
381 a specific oestrous state has led to a bias towards a spatial strategy within our female subjects: Grey
382 mouse lemurs have seasonal reproductive patterns and, in captivity, start cycling approximately one
383 month after a change from an artificial short-day period (LD 10:14; at our colony from October to
384 January) to a long-day period (LD 14:10; February to September). During the long-day period, female
385 mouse lemurs are polyoestrous with 3-4 cycles per year that can vary between 42 and 68 days in
386 length (Radespiel & Zimmermann, 2001; Wrogemann, Radespiel, & Zimmermann, 2001). During the
387 subsequent short-day period, grey mouse lemurs are anoestrous. Of the four female NHPs that reached
388 the task criterion, three started the dPAL training during the long-day period (F₂: 23rd of February; F₃:
389 7th of March; F₄: 30th of March). Due to the long training durations, each of these female subjects went
390 through at least one full oestrous cycle before reaching criterion. The fourth female NHP (F₁) started
391 and finished the dPAL training during the short-day period (18th of October – 22nd of December) while
392 being anoestrous.

393 A second effect on dPAL in mouse lemurs that is indicated by our data is an age effect. While
394 the sample size of successful NHPs is too low for inferential statistics, the clear difference in the
395 number of trials needed to reach the criterion between young and aged adults (for age classification
396 compare (Joly et al., 2014)) suggests that the number of trials needed to reach the criterion of the task
397 increases with increasing age. Since all NHP subjects had been checked for impaired vision by an

398 ophthalmologist prior to testing and only individuals with good vision were included in the study, the
399 performance difference between young and aged adult NHPs cannot be explained by visual deficits of
400 the aged subjects. If an age-effect on dPAL in mouse lemurs could be verified in a future study, this
401 would highly support their value as a *natural* and *chronic* NHP model of human brain-aging and
402 Alzheimer's disease, as which they are currently discussed (Joly et al., 2014; Verdier et al., 2015;
403 Verdier & Mestre-Francés, 2016), especially because a standardized task that assesses hippocampal
404 malfunctioning is currently lacking in mouse lemurs.

405

406 *Comparative data on dPAL in non-human mammals and humans.* The comparison of our results with
407 published data from the rodent literature on dPAL showed that, in terms of learning dynamics, mice,
408 rats, and mouse lemurs are comparably slow and that humans are considerably faster in reaching the
409 task criterion. While one would normally also expect the tested NHP to outperform the rodents, the
410 fact that rodent performance is actually *en par* with that of the tested NHP corroborates the postulation
411 that successful completion of the dPAL task in mammals relies on conserved cognitive mechanisms
412 (Nithianantharajah et al., 2015) (e.g. hippocampus-based spatial learning and/or striatum-based
413 stimulus-response learning (Delotterie et al., 2015; C. H. Kim et al., 2015; M. Kim et al., 2016; Talpos
414 et al., 2009); compare below). The humans, on the other hand, had several decisive advantages over
415 the animals tested in the task: Firstly, while animals must learn to discriminate the three items that
416 constitute the different stimulus-combination pairs, this step probably is obsolete in the human
417 subjects, due to the pictorial nature of the items (flower, airplane, spider). This is an additional reason
418 why we would recommend the utilisation of more abstract, featureless stimuli (e.g. (C. H. Kim et al.,
419 2015)) for future studies. Secondly, the human subjects had the advantage of a fast, voluntary rejection
420 of ineffective strategies as well as almost immediate rule generalization once they had learned the first
421 object-location paired-associate by trial and error. These abilities, however, require the conscious
422 expectation of the existence of an underlying rule, which is probably unique to humans.

423 Nevertheless, the post-acquisition interviews revealed that humans can also use two different
424 strategies to solve the dPAL task, a spatial one, in which each item is mapped to an absolute, correct
425 location, and a memorizing strategy, in which the correct response is learned for each stimulus-

426 combination pair without the necessity for absolute spatial mapping. The two human subjects who
427 self-reportedly chose the latter strategy could, just like the NHP M₁, be identified based on their error
428 profiles: Towards the end (last third of individual errors made), these non-spatial learners also showed
429 a clear bias for errors in trials with either SC₁ and/or SC₆ being presented (Fig. 4A). Both subjects
430 reported that they were confused by the visual similarity between SC₁ and SC₆, as it consists of
431 identical stimuli (“flower” and “spider”) presented in the same spatial order (“flower” on the left side,
432 directly adjacent to the “spider” on the right side), but differs in the belonging correct locations. For
433 rodents, a comparable analysis of the error profiles had not been available in the literature. However, a
434 dissociation between two possible learning strategies in dPAL has also been proposed for mice and
435 rats, based on pharmacologic and excitotoxic lesioning studies conducted in these species ((Delotterie
436 et al., 2015; C. H. Kim et al., 2015; M. Kim et al., 2016; Talpos et al., 2009); compare below).

437

438 *The translational value of dPAL.* As stated in the introduction, the Human CANTAB PAL and the
439 animal dPAL model different, though possibly related, psychological constructs: The human protocol
440 requires the tested subjects to recall the position of several visual stimuli on a computer display on a
441 trial unique basis and after a brief delay between stimulus presentation and retrieval (Sahakian et al.,
442 1988). In the here-described dPAL protocol, the task is acquired incrementally and in each trial a
443 choice has to be made between a simultaneously presented object-location match vs. an object-location
444 mismatch (e.g. (Horner et al., 2013)). Due to the lack of both trial uniqueness and the delayed
445 response, the dPAL paradigm cannot be seen as a model for episodic or episodic-like memory in
446 humans and animals, respectively. Nevertheless, clinical evidence for the translational value of dPAL
447 was provided by Nithianantharajah and colleagues who showed parallel cognitive deficits in mice and
448 humans (human CANTAB PAL) with genetic perturbations of the Dlg2 gene (Nithianantharajah et al.,
449 2013). There are two possible explanations for this finding: (I) Even though Human CANTAB PAL
450 and dPAL model different psychological constructs, performance in both depends on a common
451 cognitive component that is equally affected in humans and mice with Dlg2 mutations. If this is true,
452 the most obvious common link between the two paradigms would be the necessity to retrieve
453 combined visual and spatial information, a cognitive function that has also been shown to be

454 hippocampus-dependent in the absence of trial uniqueness and delay in rats using a non-CANTAB
455 protocol (Yoon, Seo, Kim, & Lee, 2012). (II) Human CANTAB PAL and dPAL do not rely on
456 homologue cognitive functions, but there is overlap in the brain areas involved in performing both
457 tasks (e.g. the hippocampal formation). Which one of the two options is true is difficult to test. To
458 avoid this general dilemma, i.e. translational problems resulting from species specific adaptations of
459 protocols initially designed for humans, a recently suggested approach is the utilization of identical,
460 highly controlled, touchscreen-based cognitive tasks designed for animal testing across all species,
461 including humans (Nithianantharajah et al., 2015). Indeed, it was shown that the same parallel
462 cognitive deficits as in the preceding study (Nithianantharajah et al., 2013) also became apparent when
463 both mice *and* humans with *Dlg2* gene mutations were tested in dPAL (Nithianantharajah et al., 2015).
464 The authors argue that using the identical task across species, from mice to humans, highly increases
465 construct validity as it is more likely that under these conditions the involved cognitive processes are
466 adequately homologous between different mammalian species, though probably more basal and
467 conserved as those assessed by more complex protocols designed for humans. Our study supports this
468 suggestion and the suitability of the dPAL protocol for broadly comparative research, as it shows for
469 the first time that the highly standardized dPAL protocol can directly be used to train a nocturnal NHP
470 (*M. murinus*) in object-location paired-associates learning. Learning performance in mouse lemurs
471 was not different from that reported in rodents (Bartko et al., 2011; Talpos et al., 2009), suggesting
472 that dPAL is based on conserved cognitive mechanisms that need to be further specified: From the
473 rodent literature, it is known that post-acquisition dPAL performance in rats is impaired after the
474 pharmacologic manipulation of the dorsal hippocampus using glutamatergic antagonists (Talpos et al.,
475 2009) or parenteral, systemic administration of NMDA antagonist or indirect dopamine agonist
476 (Talpos, Aerts, Fellini, & Steckler, 2014). In mice, genetic manipulation of the glutamatergic system
477 (TNiK^{-/-}) revealed impaired dPAL acquisition in knockouts as compared to wild type mice (Coba et
478 al., 2012) and lesions to the dorsal hippocampus led to impaired dPAL performance both during and
479 after acquisition (C. H. Kim et al., 2015). A second study using excitotoxic lesioning of the
480 hippocampus only found post-acquisition impairments in dPAL, whereas acquisition was severely
481 disrupted in animals with striatal lesions (Delotterie et al., 2015). The most likely explanation for the

482 fact that post-acquisition lesioning of the dorsal hippocampus robustly affects dPAL performance in
483 rodents (Delotterie et al., 2015; C. H. Kim et al., 2015; M. Kim et al., 2016; Talpos et al., 2009) while
484 acquisition is not (Delotterie et al., 2015; Talpos et al., 2009) or only mildly (C. H. Kim et al., 2015)
485 affected by hippocampus lesions is that intact animals acquire the task in a hippocampus-dependent
486 manner (hence the profound effect of post-acquisition lesioning) but switch to alternative (equally
487 effective) learning strategies (e.g. stimulus-response learning) if lesioning has occurred prior to
488 acquisition (Delotterie et al., 2015; C. H. Kim et al., 2015). Our results are in line with the idea that
489 two alternative strategies can be used for successful dPAL acquisition, as the error profiles in mouse
490 lemurs either show biases towards stimulus combination pairs with increased object similarity
491 (SC_1/SC_6) and short response latencies (N=1; indicative for a stimulus-response strategy) or for
492 stimulus combination pairs with increased demands on spatial processing (S_3/S_4) and long response
493 latencies (N=4; indicative for a spatial strategy). They further show that the spatial strategy, i.e. the
494 mapping of objects onto locations, is the one predominantly used for successful task completion in
495 both mouse lemurs (N=4; 80%) and humans (N=10; 83%).

496

497 **Conclusion**

498 Our study showed that the dPAL task on visuo-spatial paired associates learning originally designed
499 for rodent testing (Talpos et al., 2009) can be used successfully to train a non-human primate as well
500 as humans. This lays the foundations for the assessment of standardized paired-associates learning
501 across different primate species to track cognitive changes over aging in order to match physiological
502 profiles and behaviour in a comparative approach. To reach criterion, both the tested NHPs and
503 humans seem to rely on one of two alternative cognitive strategies: Most of the subjects tested here
504 used a strategy that includes spatial processing (suggesting a high construct validity), as intended by
505 the developers of the task (Talpos et al., 2009). Much fewer subjects used a strategy including
506 visually-guided stimulus response learning. This is in accordance with neurobiological models of
507 dPAL in rodents, in which an involvement of hippocampal and striatal regions in dPAL was found in
508 pharmacologic and excitotoxic lesioning studies (Delotterie et al., 2015; C. H. Kim et al., 2015; M. Kim
509 et al., 2016; Talpos et al., 2009). Therefore, our findings support the recent postulation that dPAL in

510 mammals relies on conserved cognitive mechanisms (Nithianantharajah et al., 2015). By
511 demonstrating for the first time that the protocol can be applied to a promising NHP model of human
512 brain-ageing, they further suggest that the highly standardized dPAL (and similar animal-testing
513 protocols) may function as unique tool for biomedical research and its translation to the clinic, due to
514 its broad applicability from rodents over NHPs to humans. Such a “reverse” approach to cognitive
515 testing can contribute to explore mechanisms of disease progression and novel therapeutic avenues in
516 psychiatric diseases, but will also provide novel insights into the evolution of intelligence in mammals
517 in general.

518

519 **Competing interests:** We have no competing interests.

520

521 **Author contributions:** DS, SA, MJ, and EZ conceived and designed the study. DS, SA, and MJ
522 conducted the experiments in the NHPs, DS conducted the experiments in humans. DS coded the NHP
523 version of the dPAL protocol and designed and coded the human dPAL protocol. DS performed the
524 data analysis and wrote the first draft of the manuscript. SA, MJ, and EZ participated in writing. DS
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526

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645

646

647 **Tables:**

648 **Table 1: Number of terminal (last third) errors separated into individual stimulus combinations**
649 **and NHPs.** The ratio given in the rightmost column represents the mean number of errors in stimulus
650 combinations with narrow spatial distance between S⁺ and item-place mismatch (SC₁, SC₃, SC₄, and
651 SC₆) divided by the mean number of errors in stimulus combinations with large spatial distance
652 between S⁺ and item-place mismatch (SC₂ and SC₅).

653

NHP	SC₁	SC₂	SC₃	SC₄	SC₅	SC₆	Ratio
M₁	118	132	42	47	70	139	0.856
F₁	74	52	107	128	56	68	1.745
F₂	149	45	127	198	75	77	2.296
F₃	231	190	469	611	372	378	1.503
F₄	126	108	253	222	117	307	2.018

654

655

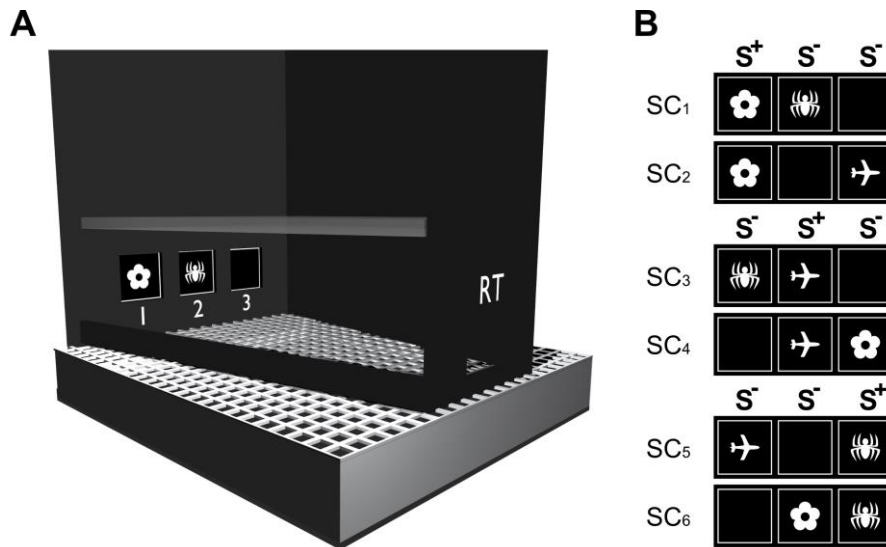
656 **Table 2: Individual median reward and response latencies of the NHPs.** Confidence intervals (CI)
657 are presented as percentile bootstrap confidence intervals based on 10000 bootstrap samples per
658 median. Effect sizes were calculated from Wilcoxon statistics as $r = z/\sqrt{N}$ with M₁ being the
659 reference and F₁-F₄ being compared to M₁. The Response/Reward ratio was calculated by dividing the
660 response latency of a given animal by the reward latency of the same animal.

661

NHP	Median response latency [s] (95% CI)	Effect size: r	Median reward latency [s] (95% CI)	Effect size: r	Response/Reward ratio
M₁	1.777 (1.776, 1.827)	-	1.117 (1.116, 1.117)	-	1.59
F₁	2.945 (2.893, 3.023)	0.80	0.916 (0.915, 0.964)	-0.33	3.22
F₂	2.589 (2.539, 2.640)	0.74	0.965 (0.965, 0.966)	-0.34	2.68
F₃	3.554 (3.503, 3.603)	0.78	1.066 (1.066, 1.067)	-0.66	3.33
F₄	4.370 (4.267, 4.469)	0.83	1.321 (1.320, 1.321)	-0.40	3.31

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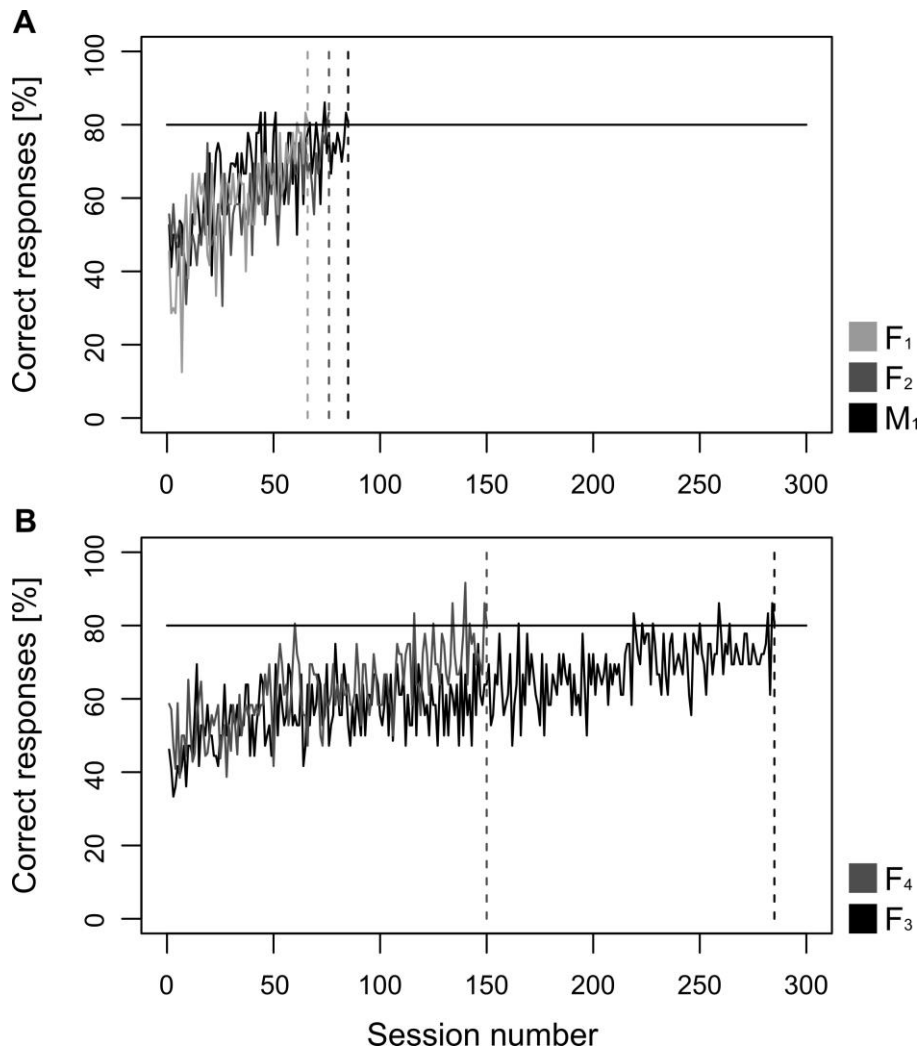
663 **Figures:**



664

665 **Fig. 1: Experimental setup and procedure.** **A** Schematic drawing of the automated Bussey-Saksida
666 Touchscreen Chamber (left sidewall and reward pump removed); **1-3** response windows 1-3; **RT**
667 entrance to the reward tray; to keep the animals from climbing, the chamber height was limited to
668 10 cm using a translucent Plexiglas lid. **B** Stimulus combinations (**SC₁-SC₆**) that were used for dPAL
669 training.

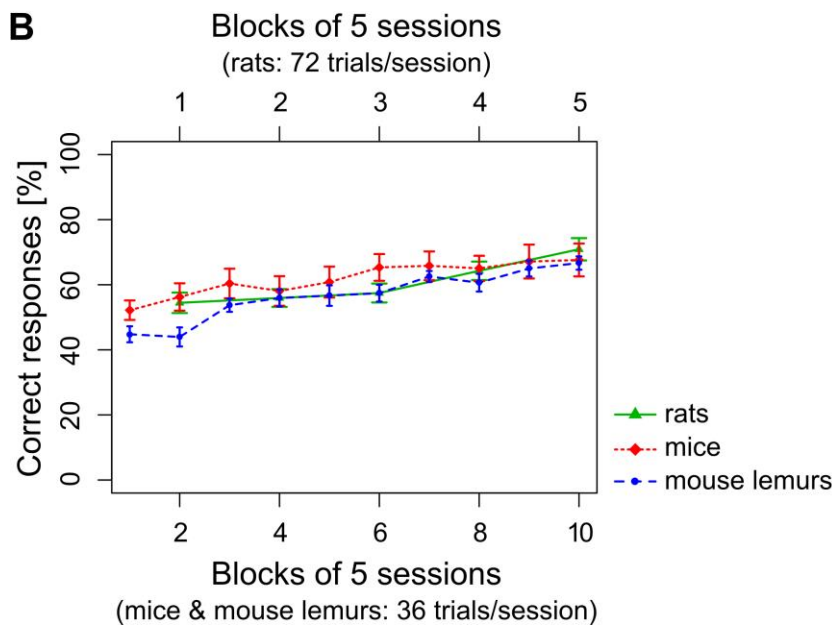
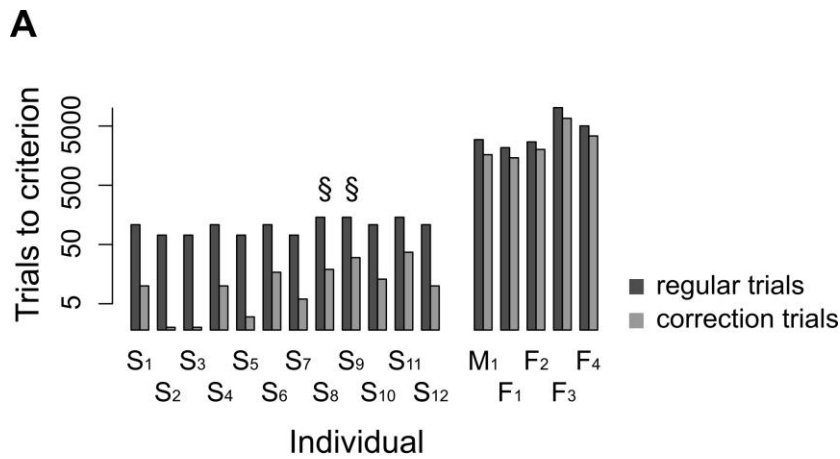
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672 **Fig. 2: Individual learning curves of the successful mouse lemurs.** **A** Individual learning curves of
 673 the three young (<4 years) adults (F₁, F₂, M₁). **B** Individual learning curves of the two aged (>7 years)
 674 adults (F₃, F₄). **A-B** The black, solid, horizontal line indicates the 80% learning criterion that had to be
 675 reached in two consecutive, complete sessions in order to finish the task; the vertical, dashed lines
 676 indicate the sessions at the end of which the criterion was reached by the respective individuals.

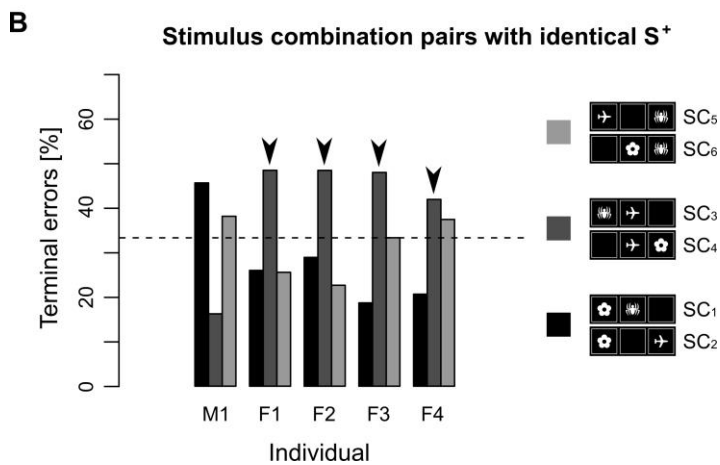
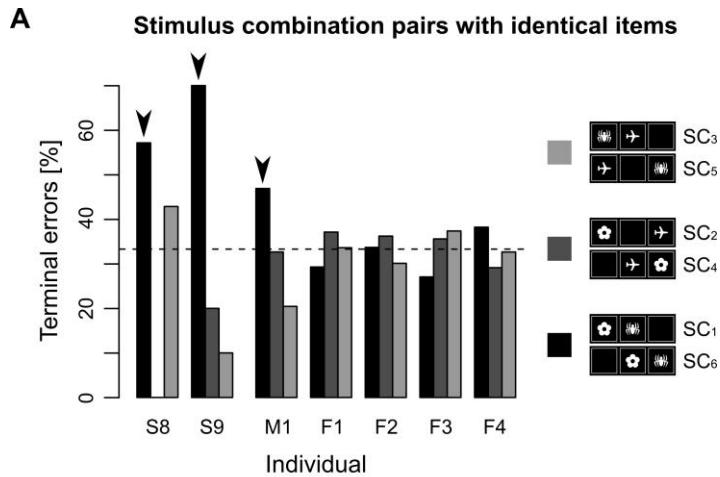
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679 **Fig. 3: Cross-species comparisons of dPAL learning performance.** **A** Comparison of the individual
 680 number of trials needed to reach the learning criterion between male, human adults (S₁-S₁₂) and mouse
 681 lemurs (M₁, F₁-F₄); please note that the ordinate is scaled logarithmically (for a non-logarithmic
 682 presentation of the human data see Fig. S5); § human subjects that self-reportedly reached the criterion
 683 by memorizing all possible stimulus combinations (SC₁-SC₆) instead of finding out the visuo-spatial
 684 rule behind the task. **B** Learning performance of the young mouse lemurs as compared to literature
 685 values for young, male Lister Hooded rats (Talpos et al., 2009) and young, male C57BL/6 mice
 686 (Bartko et al., 2011); Values are presented as group means ±SEM (N_{rats}=7; N_{mice}=7; N_{mouse lemurs}=3).

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688

689 **Fig. 4: Individual distribution of terminal errors (last third)** compared between humans that solved

690 the task by memorizing all possible stimulus combinations (S₈ [n=7] and S₉ [n=10]) and mouse lemurs

691 (M₁ [n=548], F₁ [n=485], F₂ [n=671], F₃ [n=2251], F₄ [n=1133]). **A** Error distributions separated into

692 stimulus combination pairs with identical items (SC₁/SC₆, SC₂/SC₄, SC₃/SC₅). The two human subjects

693 (S₈ and S₉) showed a clear overrepresentation of terminal errors in SC₁/SC₆ (black arrow heads). In

694 *M. murinus*, a similar pattern with a significant overrepresentation of terminal errors in SC₁/SC₆ (black

695 arrow head) and medium effect size ($\varphi_c=0.229$) was found in M₁; dashed line = chance level (33.3%).

696 **B** Error distributions separated into stimulus combination pairs with identical S⁺ (SC₁/SC₂, SC₃/SC₄,

697 SC₅/SC₆). In *M. murinus*, F₁-F₄ showed a significant overrepresentation of terminal errors in SC₃/SC₄

698 (black arrow heads) with medium effect sizes ($\varphi_c=0.19$ to 0.25); dashed line = chance level (33.3%).

699