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Prolactin and fMRI response to SKF38393 in the baboon

Background: This study's goal was to provide dose-response data for a dopamine agonist in the baboon using standard methods (replicate measurements at each dose, across a range of doses), as a standard against which to subsequently validate a novel pharmacological MRI (phMRI) method. Dependent variables were functional MRI (fMRI) data from brain regions selected *a priori*, and systemic prolactin release. Necessary first steps included estimating the magnitude and time course of prolactin response to anesthesia alone and to various doses of agonist. These first steps ("time course studies") were performed with three agonists, and the results were used to select promising agonists and to guide design details for the single-dose studies needed to generate dose-response curves. **Methods:** We studied 6 male baboons (*Papio anubis*) under low-dose isoflurane anesthesia after i.m. ketamine. Time course studies charted the changes in plasma prolactin levels over time after anesthesia alone or after an intravenous (i.v.) dose of the dopamine D₁-like agonists SKF82958 and SKF38393 or the D₂-like agonist pramipexole. In the single-dose dopamine agonist studies, one dose of SKF38393 (ranging from 0.0928 – 9.28 mg/kg, N=5 animals) or pramipexole (0.00928 – 0.2 mg/kg, N=1) was given i.v. during a 40-minute blood oxygen level dependent (BOLD) fMRI session, to determine BOLD and plasma prolactin responses to different drug concentrations. BOLD response was quantified as the area under the time-signal curve for the first 15 minutes after the start of the drug infusion, compared to the linearly predicted signal from the baseline data before drug. The ED₅₀ (estimated dose that produces 50% of the maximal possible response to drug) for SKF38393 was calculated for the serum prolactin response and for phMRI responses in hypothalamus, pituitary, striatum and midbrain. **Results:** Prolactin rose 2.4- to 12-fold with anesthesia alone, peaking around 50-90 minutes after ketamine administration and gradually tapering off but still remaining higher than baseline on isoflurane 3-5 hours after ketamine. Baseline prolactin level increased with age. SKF82958 0.1mg/kg i.v. produced no noticeable change in plasma prolactin concentration. SKF38393 produced a substantial increase in prolactin release that

peaked at around 20-30 minutes and declined to pre-drug levels in about an hour.

Pramipexole quickly reduced prolactin levels below baseline, reaching a nadir 2-3 hours after infusion. SKF38393 produced clear, dose-responsive BOLD signal changes, and across the four regions, ED_{50} was estimated at 1.6-7.7mg/kg. **Conclusions:** In the baboon, the dopamine D_1 receptor agonist SKF38393 produces clear plasma prolactin and phMRI dose-response curves. Variability in age and a modest sample size limit the precision of the conclusions.

Introduction

We recently described a new pharmacological MRI (phMRI) method designed to estimate a dose-response curve in a single imaging session (Black, Koller & Miller, 2013). We sought to validate that method using specific dopamine receptor agonists in the baboon, a primate species with a large enough brain to allow using functional MRI (fMRI) methods commonly used in humans (Black et al 2009). However, limited data have been available on dose-response curves for fMRI responses to dopamine agonists in primate brain. More data are available on the systemic prolactin response to some dopamine agonists. The goal of the present work was to use a traditional approach to characterize the dose-response curves for regional fMRI signal and prolactin responses in baboon to at least one pharmacologically specific dopamine agonist.

Primate phMRI studies, and before that *ex vivo* and positron emission tomography studies of regional cerebral metabolism and blood flow, have been reported for levodopa and several dopamine agonists (Black et al 2000, 2002; Chen et al 1997; Herscovitch 2001; Hershey et al 2000, 2003; Perlmuter, Rowe & Lich 1993; Trugman and Wooten 1996; Trugman and James 1992, 1993; Zhang et al 2000). However, rarely have phMRI dose-response curves been reported for drugs of any kind in primates (briefly reviewed in Black et al 2010).

Prolactin is a hormone best known for its ability to promote lactation in response to the suckling stimulus of hungry young mammals, but it plays a role in many biological functions (Freeman et al 2000). Prolactin is produced principally by the anterior pituitary gland, and also within the central nervous system, the immune system, the uterus and the mammary gland. Pituitary prolactin secretion is mediated in large part by dopamine synthesized by the tuberoinfundibular dopaminergic neurons of the arcuate nucleus through D₂ receptors (Freeman et al 2000; Jaber et al 1996; Ben-Jonathan and Hnasko 2001). Dopamine is the principal prolactin inhibiting factor and increasing dopamine directly by giving dopamine i.v. reduces plasma prolactin concentration (LeBlanc et al 1976; Norman et al 1980; Freeman et al 2000; Ben-Jonathan and Hnasko 2001). D₁ receptor activation also increases prolactin levels most likely by inhibiting the D₂ pathway (Curlewis et al 1993; Colthorpe et al 1998; Durham et al 1998; Freeman et al 2000).

SKF82958 is a full agonist at dopamine D₁-like receptors, though there is some debate about full vs. partial agonism (Gilmore et al 1995; Bergman et al 1996). SKF82958 produces dose-responsive effects on brain activity (Black et al 2000), but its effects on prolactin secretion are not well described. Pramipexole is a D₃-preferring D₂-like dopamine agonist (Mierau 1995; Mierau et al 1995; Piercey 1998) that produces regionally specific effects on brain activity (Black et al 2002) and reduces prolactin secretion in humans (Schilling et al 1992; Samuels et al 2006a). For both of these drugs there is strong evidence that they affect regional cerebral blood flow (rCBF) via changes in neuronal activity and not via direct vascular effects (Black et al 2000, 2002). In fact, with few exceptions (such as ergot derivatives), synthetic dopamine agonists generally do not uncouple brain blood flow from brain metabolism, *i.e.* are not expected to affect the BOLD signal except through neuronal effects (discussed in Black et al 2000, 2002).

SKF38393 is a partial D₁ agonist that increases prolactin levels and changes regionally specific brain blood activity (Dixon et al 2001, Engber et al 1993, Trugman & James 1993, Morelli et al 1993). There is little information on fMRI or rCBF effects of SKF38393, but it does affect neuronal function and regional cerebral metabolism via dopamine D₁ receptors (Trugman and James, 1992). SKF38393 has been shown to increase prolactin anywhere from 3- to 40-fold in several species including humans. Its effect on prolactin levels is rapid, peaking about 15-20

minutes after parenteral administration in the rat or sheep (Cocchi et al 1987, Tanimoto et al 1987, Curlewis et al 1993), and prolactin levels return to baseline in about an hour. When given orally, the delay is 60-150 minutes, returning to baseline within two to three hours (in humans; Fabbrini et al 1988). Some, but not all, studies report dose-response characteristics with regard to prolactin secretion (Salama 1986, Cocchi et al 1987, Tanimoto et al 1987, Fabbrini et al 1988, Curlewis et al 1993, Saller and Durham et al 1998).

Here we report preliminary data from male baboons on the timing and magnitude of prolactin response to anesthesia alone (N=6), to SKF82958 (N=1), to SKF38393 (N=5), and to pramipexole (N=1). Then we compare the prolactin and regional BOLD (blood oxygen level dependent) fMRI responses to SKF38393 (N=5).

Materials & Methods

Subjects

The study was approved by the Washington University Animal Studies Committee (protocol # 20090112). We studied six healthy male olive baboons (*Papio anubis*). This species was chosen with a goal of later translation to human studies; specifically, baboon anatomy and neuroendocrine responses are similar to humans', and their brain volume is big enough to allow neuroimaging methods borrowed from human fMRI (Black et al 2009). Three were fully adult (age 6.8-14 years) and three were juveniles (age 4 years). In captive male baboons, puberty occurs at about 3.5 years of age (VandeBerg 2009, p. xxi), and the testes had descended in all subjects before these studies began.

Overall approach

We report results from three types of studies: time-course studies with anesthesia alone, time-course studies with a dopamine agonist, and single-dose dopamine agonist studies. To minimize drug sensitization and blood loss, no two studies in a given animal occurred less than two weeks apart.

Time course studies

Anesthesia alone

First we charted the effects of ketamine and isoflurane on prolactin secretion over time without administering a dopamine agonist, since both these anesthetic agents increase plasma prolactin levels (Wickings & Nieschlag 1980; Puri et al 1981; Crozier et al. 1994; Hergovich et al 2001; Rizvi et al 2001). We took a blood sample as soon as possible after giving ketamine, with the expectation that circulating prolactin concentrations would not change for >20 minutes after i.m. ketamine (Rizvi et al 2001). The goal was to determine when the effects of ketamine and/or isoflurane on prolactin release would reach an approximate steady state. Blood samples were taken in 4- to 20-minute intervals for up to 5 hours.

With dopamine agonist

Time course studies were also done for the dopamine agonists to provide initial information on whether the agonist produced a detectable effect on prolactin release in this model and to determine a reasonable sampling time for the later single-dose studies.

115 In these studies, a 40-minute BOLD fMRI scan began at least 2 hrs after i.m. ketamine and
116 glycopyrrolate. Sedation from i.m. ketamine is long gone by this time, and a prolactin increase
117 after ketamine in rhesus monkeys had returned to baseline by 60 minutes (Quadri et al 1978).
118 A syringe pump delivered the dopamine agonist i.v. to anesthetized subjects at a constant rate
119 beginning at 15 min and ending at 20 min into the scan.

Five animals received varying doses of SKF38393, each dose on a different day. The order of doses was random in each subject. The doses used were 0, 0.0928, 0.2, 0.431, 0.928, 2.0, 4.31, and 9.28 mg/kg; not all subjects received all doses. A sixth animal received pramipexole at doses of 0, 0.00928, 0.0431, 0.0928 and 0.2 mg/kg.

124 In these single-dose studies, blood samples for prolactin levels were taken just before dopamine
125 agonist administration and again 20-30 minutes after drug infusion ended (for pramipexole, 60
126 minutes after infusion). The sampling time for each animal was based on the results of the time
127 course studies.

129 Animals were fasted overnight before anesthesia. After ketamine 6-12mg/kg i.m. and
130 glycopyrrolate (0.013-0.017 mg/kg i.m., to reduce secretions), orotracheal intubation was
131 performed via direct laryngeal visualization. Anesthesia was maintained with isoflurane and
132 oxygen beginning 10-30min. after ketamine. An i.v. catheter was placed in one or both legs for
133 drug delivery and withdrawing blood samples. Isoflurane delivery rate was adjusted to maintain
134 the concentration of isoflurane in expired air at the lowest level that would reliably maintain
135 sedation during study sessions. This concentration was measured by a Surgivet V9400 monitor
136 (Smiths Medical, Norwell, MA), and the optimal concentration ranged from 0.95%-1.45% across
137 animals.

SKF82958 (catalog # C130, Sigma-Aldrich, St Louis, MO) and SKF38393 (catalog # D047, Sigma-Aldrich, St Louis, MO) were mixed fresh the day of the study, the pH was adjusted with NaOH, then the solution was filter sterilized (0.22 μ m) and stored on ice in a light-tight container until use. SKF82958 was prepared with sterile 0.9% saline at 1.0 mg/ml and the pH adjusted to 6.0-6.5. SKF38393 was prepared with sterile water from 1.0 to 7.3 mg/ml and the pH adjusted to 5.5-6.0. Pramipexole (a gift from Boehringer-Ingelheim Pharmaceuticals, Inc., Ridgefield, CT) was mixed with sterile saline at 1mg/ml and filter sterilized before use. Some pramipexole

146 solution was kept in aliquots at -80°C for later use.

147 **Plasma prolactin analysis**

148 Prolactin concentration in plasma or serum was measured via radioimmunoassay (University of
149 Wisconsin National Primate Research Center, Madison, Wisconsin). The inter-assay coefficient
150 of variation was 7.89%, and the intra-assay coefficient of variation was 4.09%.

151 **MRI sequences and initial processing**

152 All imaging was done on a Siemens Trio 3T scanner (Erlanger, Germany) with boosted gradient
153 coils. To facilitate image registration, an MP-RAGE sequence was used to acquire a T1-weighted
154 structural image of the head, flip angle 7° , echo time (TE) 3.51ms, inversion time 1000ms, FOV
155 $192 \times 192 \text{mm}$, 192 slices, and repetition time (TR) 1900ms; and a 3D turbo spin echo sequence
156 was used to acquire a T2-weighted structural image, flip angle 120° , TE 604ms, field of view
157 $256 \times 256 \text{mm}$, 192 slices, and TR 3200ms. Voxel size was approximately $(0.8 \text{mm})^3$ for both
158 structural images.

159 Functional images were acquired using a blood oxygenation level-dependent (BOLD) contrast-
160 sensitive echo-planar sequence with flip angle 90° , TE 30ms, field of view $384 \times 384 \text{mm}$, 32
161 slices, voxel size $(2.5 \text{mm})^3$, and TR 2160ms, with interleaved slice acquisition. Over a period of
162 40 minutes, 1111 volumes (frames) were acquired; the first 4 frames were discarded to ensure
163 steady-state magnetization.

164 Functional images from each subject were preprocessed to reduce artifacts, including correction
165 for intensity differences due to interleaved acquisition, interpolation for slice time correction,
166 correction for head movement, and alignment to atlas space (Black et al., 2001). Image intensity
167 was not adjusted on a frame-by-frame basis, but the entire 40-minute acquisition was scaled
168 linearly to a mean (across frames) whole-brain modal value of 1000.

169 **Volumes of interest**

170 Volumes of interest (VOIs) were defined by hand on each animal's MP-RAGE (structural image)
171 for the following regions of brain: hypothalamus, pituitary, striatum, and midbrain. The
172 hypothalamus and pituitary were chosen because of the potential relevance to prolactin release,
173 and the striatum and midbrain to provide comparisons to prior blood flow, metabolic, and BOLD
174 pharmacological activation studies of dopamine agonists. The regions were drawn with reference
175 to Davis & Huffman (1968) by one author (BM) with over 20 years' experience in neuroanatomy
176 (including delineating subnuclei in rodent thalamus and tract tracing over the entire length of the
177 primate brain). All regions were drawn before any regional time-signal curves were examined.
178 The VOIs were transferred to the MRI by a validated cross-modality image registration method
179 (Black et al., 2001).

180 **Determination of ED_{50}**

181 The mean prolactin plasma concentration response to each dose of SKF38393 in the single-dose
182 studies was plotted against the logarithm of the dose. A sigmoid E_{max} model (Holford and Sheiner,
183 1982) was fit to the data using a Markov chain Monte Carlo (MCMC) method (Bretthorst, 1988;
184 Bretthorst and Marutyan, 2012). To improve sampling across the logarithmic abscissa, a variable
185 $Q = \log_{10}(\text{ED}_{50})$ was defined and the MCMC method was used to estimate parameters in the

186 equation

187
$$E(D) = E_0 + E_{\max} \cdot D / (D + 10^Q),$$

188 where D = dose of SKF38393 in mg/kg, E_0 = response at baseline ($D=0$), and $E(D)$ = response
189 (effect) at dose D . The response at each dose was defined as post-drug minus pre-drug prolactin
190 concentration on that same day (hence the nonzero baseline response E_0).

191 Separate dose-response curves and ED_{50} estimates were derived from mean BOLD (fMRI)
192 responses to SKF38393 and pramipexole in each VOI. The BOLD response at a given dose was
193 defined as the area under the BOLD signal curve (AUC) from 15 to 30 minutes after drug, after
194 accounting for the linear trend in the first 15 minutes of data:

$$AUC = \int_{15}^{30} BOLD(t) - (mt + b) dt,$$

195

196 where time t is in minutes, $BOLD(t)$ is the BOLD signal at each time point, and $y = mt + b$ is the
197 least-squares line fit to the first 15 minutes of BOLD signal data.

198 **Results**

199 **Baseline prolactin levels**

200 Baseline prolactin levels were measured from the blood sample taken as soon as possible after the
201 subject was given ketamine. The delay between ketamine administration and the first blood
202 sample ranged from 6 to 24 minutes. These data showed no clear change in prolactin levels
203 during this time period. For example, one subject's first sample was taken as soon as eight
204 minutes or as late as 24 minutes after ketamine (a total of 11 baseline samples were examined for
205 this subject). Prolactin levels were similar across these time points (data not shown). The other
206 subjects showed a similar pattern. Thus, changes in plasma prolactin levels due to anesthetics
207 appear to occur only after this time period.

208 Baseline levels of plasma prolactin were positively correlated with age ($r = 0.96$, $p < 0.01$;
209 Table 1). The oldest animal, at 14 years of age, had five-fold higher baseline levels of prolactin
210 than any of the other subjects, but the correlation with age remained significant even excluding
211 this subject. The three juveniles had the lowest baseline measurements.

212 **Effect of anesthesia alone on prolactin release**

213 Anesthesia increased prolactin in all 6 animals ($p=.03$, 2tailed binomial distribution), with a peak
214 concentration from 2.4-fold to 12-fold higher than at baseline ($p<.07$, 2tailed paired t test). The
215 three juveniles had the lowest increase (Table 1).

216 Prolactin levels peaked between 50 to 90 minutes after ketamine. Prolactin levels declined
217 partially and leveled off somewhat around 100-120 minutes (Fig. 1). They continued to decline
218 but remained well above baseline levels for 3-5 hours (last measurement, median 208min) in all
219 animals ($p=.03$, 2-tailed binomial distribution; $p<.07$, 2-tailed paired t test). Some, but not all, of
220 the time course profiles appeared to show a small, secondary increase in prolactin after 100
221 minutes. A similar post-decline surge has been recorded in other studies with ketamine (Rizvi et

222 al 2001) and isoflurane (Reber et al 1998).

223 Prolactin release with anesthesia was greater in the adults than in the juveniles (Fig. 2; $p=0.045$).

224 **Time course studies with dopamine agonists**

225 Subsequent experiments attempted to detect an effect from the experimental drug on prolactin
226 release, and to define an appropriate time for sampling. SKF82958 at 0.1mg/kg produced no apparent
227 change in prolactin levels, which continued to decline over time in a pattern that was
228 indistinguishable from the anesthesia-only condition (Fig. 3).

229 By contrast, the partial agonist SKF38393 appeared to produce a substantial increase in prolactin
230 (Fig. 4), including in the same adult whose non-response to SKF82958 appears in Fig. 3.
231 Prolactin levels increased as soon as five minutes after the drug infusion, peaked within 20-30
232 minutes, then declined more gradually. The peak time was unique for each animal but
233 was 20-30 minutes after drug; this time determined when that animal's blood
234 sample was to be taken after drug infusion during the single dose studies.

235 Prolactin declined shortly after pramipexole administration in the single subject tested, with a
236 substantial decline by 60 minutes and reaching a nadir about three hours later (Fig. 5). Note that
237 the prolactin concentrations dropped to well below baseline level.

238 **Single-dose studies**

239 ***SKF38393 and prolactin***

240 The single dose studies showed a dose-related effect of SKF38393 on prolactin (Fig. 6). Two
241 adults (S, E) showed the most consistent and largest increases in prolactin, resulting in over a 5-
242 fold increase with the larger doses compared to pre-drug levels. One adult (D) and the two
243 juveniles had more modest and inconsistent increases, not exceeding a 2-fold increase over pre-
244 drug levels.

245 The [PRL] increase with drug was not monotonic. Of course, this comparison involves results
246 obtained on different days, and these results differed from the time course data, in which a clear
247 dose-response effect was visible when different doses were given within the same session
248 (Fig. 4). Fig. 6 plots results as the difference in prolactin concentration from the pre-drug levels,
249 but similar variability was seen when the values are plotted as percent of pre-drug levels or
250 absolute concentrations of prolactin.

251 The group median dose-response curve for prolactin release following BOLD decreased at the
252 highest dose, so strictly the sigmoid E_{\max} model may not apply. However, recognizing this
253 limitation, the model fit well to the remaining data points (0-4.31mg/kg) with an ED_{50} of
254 1.3mg/kg (Fig. 7).

255 ***SKF38393 and BOLD***

256 There was a clear effect of SKF38393 on BOLD signal in midbrain at the higher doses (Fig. 8;
257 Fig. 9). BOLD signal in pituitary decreased with increasing dose, but it increased in the other
258 three regions (Fig. 10). The regional responses to the agonist, by dose, were best fit to the

sigmoid E_{\max} model with ED_{50} s of: hypothalamus, 1.59mg/kg; midbrain, 2.25mg/kg; pituitary, 2.74mg/kg; and striatum, 7.70mg/kg.

261 ***Pramipexole***

262 The one animal given pramipexole (subject T, juvenile) showed a decrease in prolactin
263 concentration with the agonist at all doses tested (Table 2). The BOLD responses from this
264 animal are also given in Table 2.

265 **Discussion**

266 **Anesthetic effects on prolactin**

267 Our results with the anesthetics ketamine and isoflurane are very similar to those of previous
268 studies, showing an initial rise in prolactin levels followed by a gradual decline (Reber et al 1998;
269 Puri et al 1981; Rizvi et al 2001; Hergovich et al 2001). Prior studies suggest that isoflurane may
270 produce a larger prolactin response than ketamine, though the data are from different species and
271 sex. Isoflurane in human females has shown a 9 to 12-fold increase in prolactin (Reber et al 1998;
272 Marana et al 2003), while ketamine produced a 2- to 4-fold increase in male macaques after a
273 single injection (Puri et al 1981; Rizvi et al 2001). A study in adult baboon females found no
274 significant difference in PRL release between constant-rate i.v. ketamine and inhaled halothane,
275 both beginning after i.m. ketamine (Walker et al 1987).

276 However, we saw a substantial range in the magnitude of the increase in our baboons, with the
277 lowest being 2.4-fold and the highest 12-fold. Five of the six subjects had an increase of prolactin
278 from anesthetics alone that ranged from 2.4-fold to 4.7-fold compared to baseline values,
279 suggesting that isoflurane was not producing as dramatic an effect in our male baboons as it does
280 in human females. In this study, we cannot reliably separate the effects of the two anesthetics, but
281 since ketamine's anesthetic effects are largely gone an hour after i.m. administration (discussed in
282 Black et al 1997), the prolactin effects after 2-5 hours likely are primarily attributable to
283 isoflurane. However, even this conclusion must be tempered by the caveat that drug effects on
284 prolactin release are somewhat delayed.

285 The blood samples taken as soon as practical after ketamine administration suggested that
286 prolactin levels did not begin to increase significantly until > 20 minutes after ketamine, though
287 we necessarily lacked pre-ketamine data for comparison.

288 **Dopamine agonist effects on prolactin**

289 Our time course results with SKF38393 are similar to those previously published, with a rapid
290 increase in prolactin levels after drug administration (Tanimoto et al 1987). In some instances we observed
291 an increase in as little as five minutes after drug infusion. Prolactin levels peaked by 20 to 30 minutes,
292 followed by a gradual decline (Tanimoto et al 1987; Curlewis et al 1993; Cocchi et al 1987).

293 Prolactin responses generally increased with higher doses of SKF38393, but in 3 animals the
294 greatest response occurred at 2 or 4.31 mg/kg rather than at the highest dose, 9.28mg/kg. This
295 may simply be due to variability in the single-dose data as discussed below. Alternatively, the
296 physiological response of prolactin release may have an inverted U shape. Resolving this
297 question would require higher doses of drug and more subjects. We ignored the data from the
298 highest point in order to estimate an ED_{50} for prolactin response, but we recognize the limitations

299 in this approach.

300 The only previous data we found for prolactin response to the D₁ agonist SKF82958 was from an *in vitro*
 301 study (Burris et al 1991). Based on its action on D₁ receptors and previous data from SKF38393, we
 302 predicted that SKF82958 would stimulate prolactin release. At the dose used in the present study,
 303 SKF82958 produces changes in regional cerebral blood flow (Black et al 2000), but there was no clear
 304 evidence of a change in prolactin release after this dose. One speculative explanation might be that D₁ full
 305 agonists do not affect prolactin release, while the partial agonist SKF38393 increases prolactin release by
 306 its relative antagonism in the setting of intrinsic dopamine release.

307 The results from our pramipexole data are similar to those reported for human studies. We see a
 308 dose-dependent reduction in prolactin levels (Schilling et al 1992) lasting for four hours or more
 309 after a single dose, with the lowest values seen several hours after administration (Schilling et al
 310 1992; Porta et al 2009; Hood et al 2010).

311 **Variability in the single-dose studies of prolactin response to SKF38393**

312 Our time-course studies suggested a clear dose-response effect from SKF38393 on plasma prolactin levels
 313 (see Fig. 4, lower panel), in that more drug produced more prolactin. Most other studies report a similar
 314 effect (Tanimoto et al 1987, Fabbri et al 1988, Curlewis et al 1993, Cocchi et al 1987, Saller and Salama
 315 1986, Durham et al 1998). However, as noted above, this relationship was less pronounced in the single-
 316 dose studies, and possible reasons for this include the following.

317 The single-dose studies were conducted on separate days, and the physiological response could
 318 vary for that reason, even though they were expressed in relation to the pre-drug levels. The pre-
 319 drug levels varied from one session to another by as much as four-fold for a single animal. This
 320 variability was present despite our efforts to maintain the expired isoflurane at a consistent level
 321 throughout the study. With the small number of subjects studied, deviations from a monotonic
 322 increase may reflect merely noise in the data.

323 Another potential source of variability is that a single post-drug sample, even at a consistent time,
 324 may not always catch the maximal effect of the drug on prolactin concentration. Also, though we
 325 attempted to maintain a similar time schedule (a two hour delay from the time we first gave
 326 ketamine to the start of the BOLD scan), that varied somewhat due to factors not under our
 327 control. Thus, there are multiple factors that could contribute to the variability we saw in the
 328 single-dose data.

329 **Age effects on prolactin**

330 Our data suggest that baboons may have age-related differences in basal plasma prolactin levels.
 331 Our sample size is too small to reach a firm conclusion in this respect. However, recently
 332 presented data from a large group of male *P. anubis* baboons showed that serum prolactin does
 333 increase with age, peaking at around 13 years; fully adult males have significantly higher serum
 334 prolactin concentrations than four-year-old males (Phillips-Conroy et al., 2013). A report by
 335 Kondo et al (2000) showed that prolactin levels increase about 3-fold (on average) with age in
 336 male chimpanzees. However, this report does not specify how long the blood sample was taken
 337 after administering ketamine and the dopamine D₂-like antagonist droperidol, and both of these
 338 agents increase prolactin (Puri et al 1981; Hayashi and Tadokoro 1984; Schettini et al 1989; Rizvi
 339 et al 2001; Hergovich et al 2001). For comparison, human males have no significant
 340 differences in prolactin levels from prepubescence to adulthood (Guyda and

Friesen 1973; Aubert et al 1974; Lee et al 1974; Ehara et al 1975; Finkelstein et al 1978).

The fully adult animals also showed a greater prolactin response to the anesthetics and to SKF38393 (Table 1, Fig. 2, Fig. 6).

BOLD responses to SKF38393

Noting the caveats above, the estimated prolactin ED₅₀ for SKF38393 was 1.3mg/kg. It seems relevant that the VOI with the closest BOLD ED₅₀ value to this was hypothalamus, with an ED₅₀ of 1.59mg/kg.

Conclusions

In the baboon, the D₁ agonist SKF38393 increased plasma prolactin levels, and caused dose-related changes in BOLD signal in several brain regions, hypothalamus, pituitary and midbrain being more sensitive than striatum. From limited data, the D₂-like agonist pramipexole produced a decline in prolactin levels, and the D₁ agonist SKF82958 did not affect prolactin release.

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Figure 1

Plasma prolactin levels over time with anesthesia alone

Two time course studies of the effect of anesthesia alone on prolactin plasma concentrations in two adults. The green line shows the period of isoflurane inhalation. The orange arrow marks when the ketamine was given.

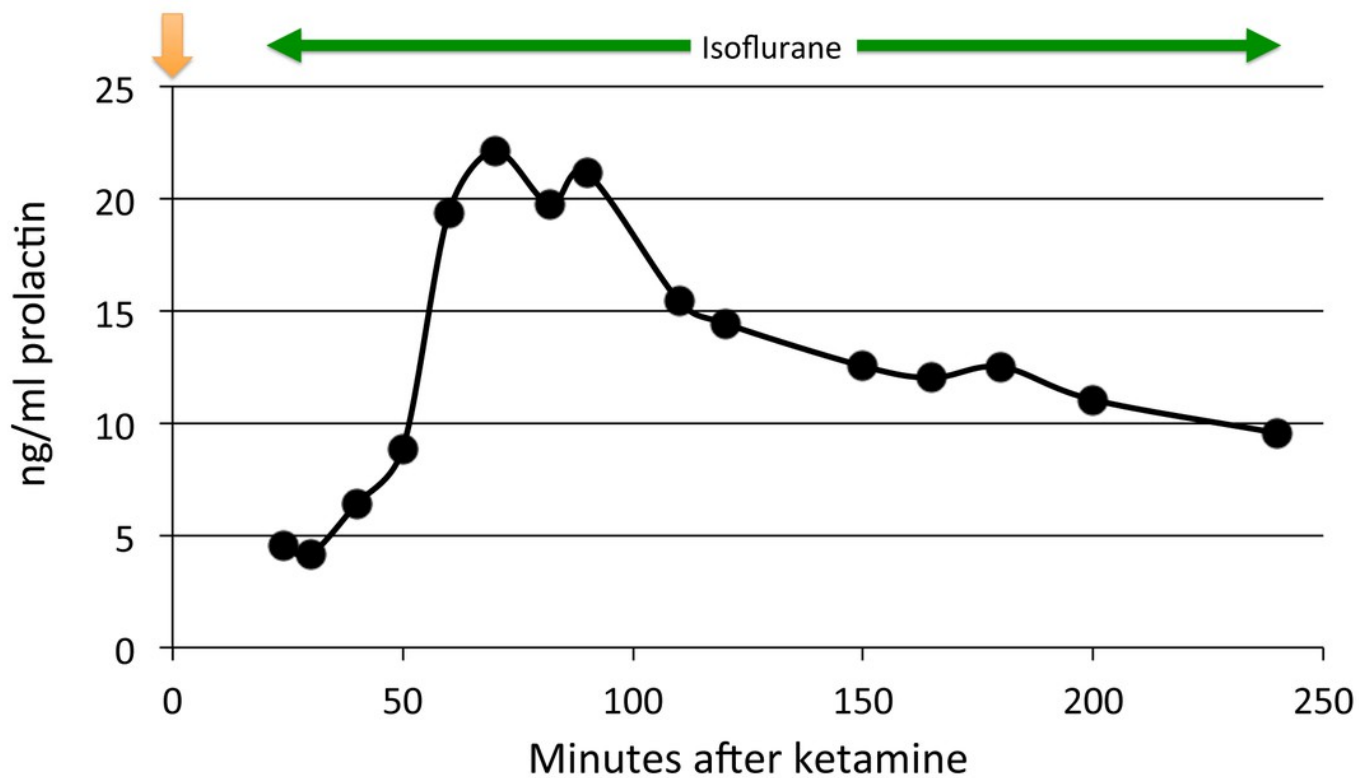
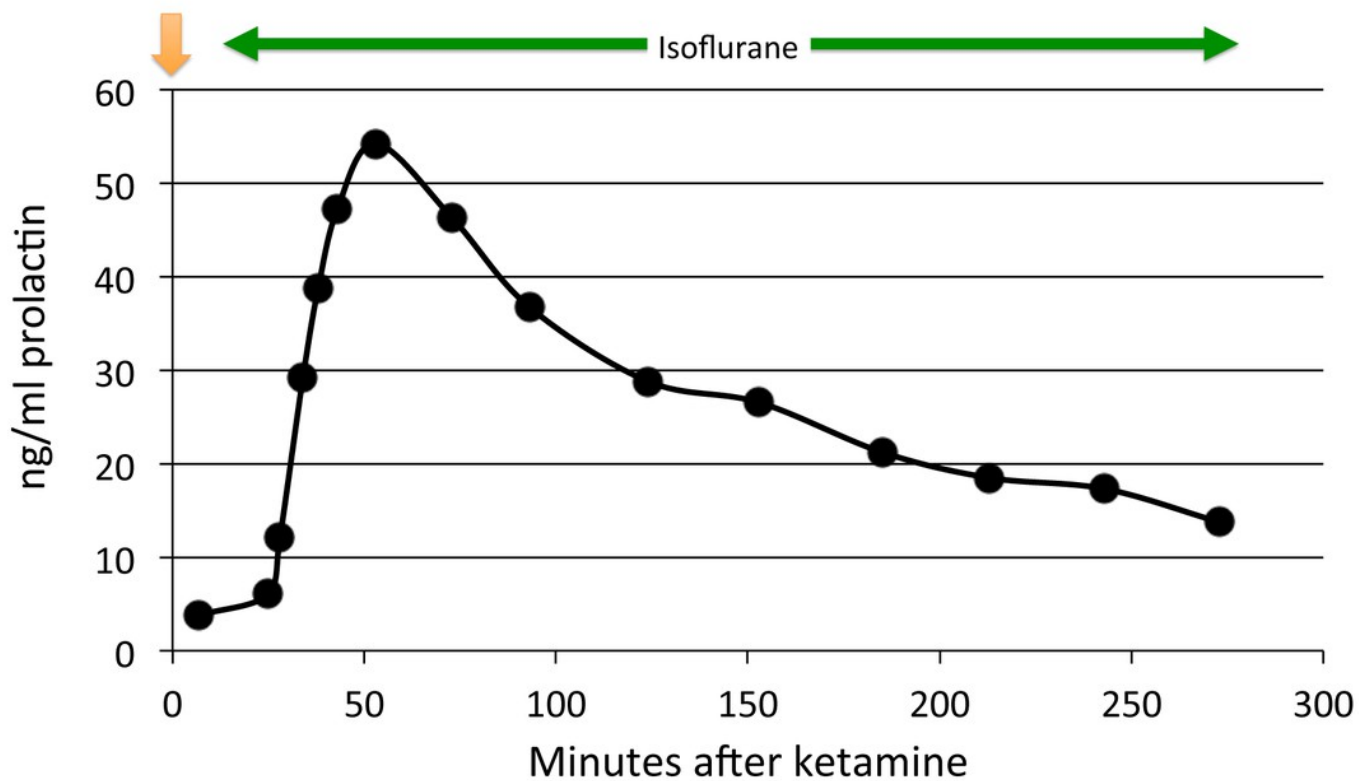


Figure 2

Age-related differences in prolactin secretion with anesthesia

We performed an ANOVA with age group as a between-subject factor and time (in 20-minute bins) as a repeated measure. Every animal had data out to at least the 121-140 minute bin.

Three missing values were imputed by averaging the previous and following levels in that animal (these two values differed by only 24%, 13%, and 0%), and in one animal the first sample was at 21' and was treated as if it had been drawn at 20'. There was a significant difference between juveniles and adults in the [PRL] response to anesthesia (age group \times time interaction $F_{6,24}=7.350$, $p=.045^*$; main effect of age group $F_{1,4}=4.103$, $p=.113$; main effect of time $F_{6,24}=12.799$, $p=.018^*$). * =Greenhouse-Geisser correction for nonsphericity

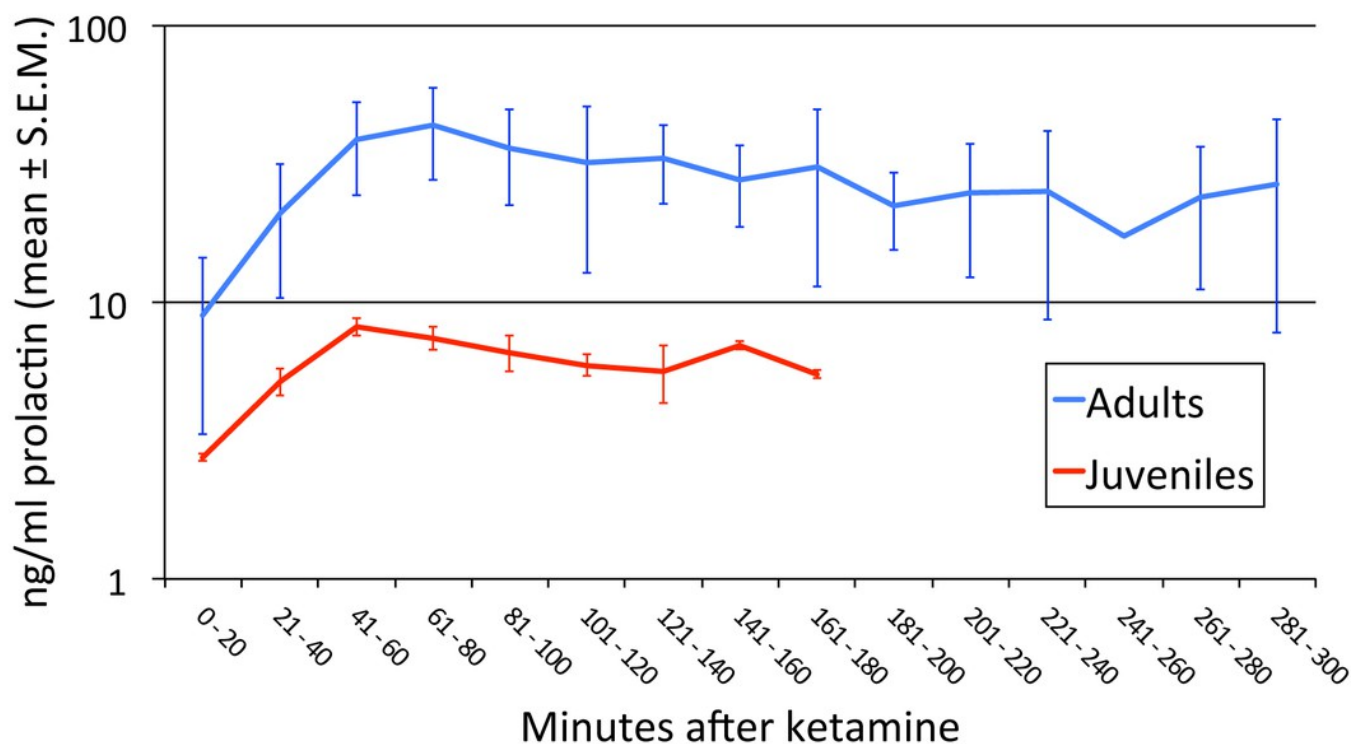


Figure 3

SKF82958 effects on plasma prolactin

Data from one of the adult animals. A single dose of 0.1 mg/kg was given at 125 minutes after ketamine (blue line).

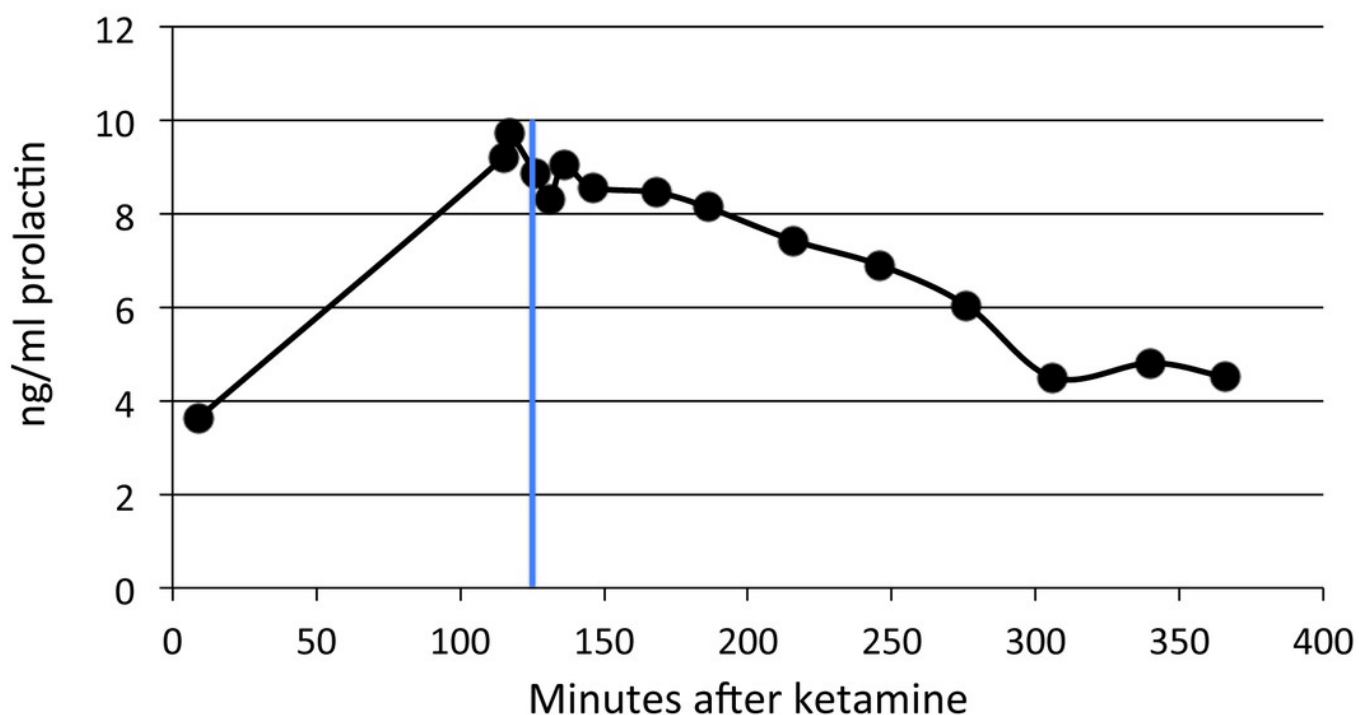


Figure 4

Time course of SKF38393 effects on plasma prolactin

One animal (top) was given a single dose of 2.0 mg/kg of SKF38393 125 minutes after ketamine. Another animal (bottom) was given successive doses of 1.0, 2.0 and 5.0 mg/kg of SKF38393 at 130, 197 and 264 minutes after ketamine (blue lines).

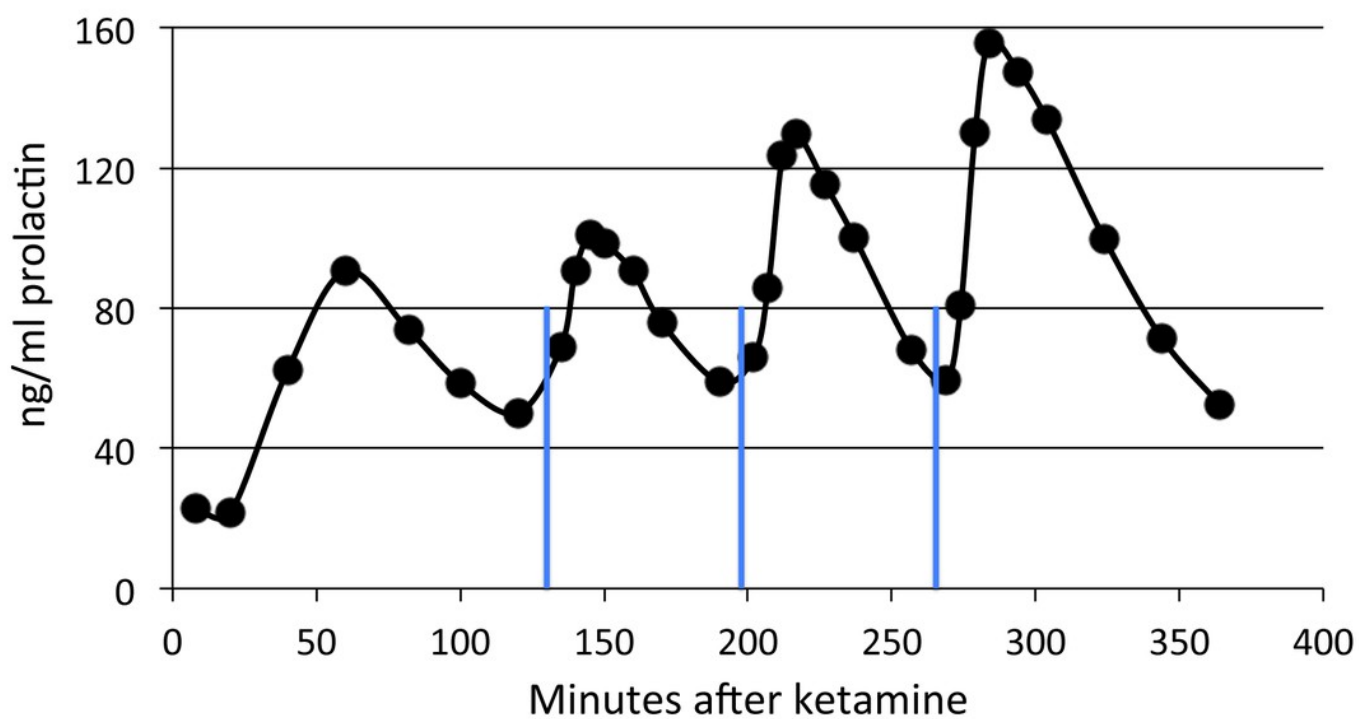
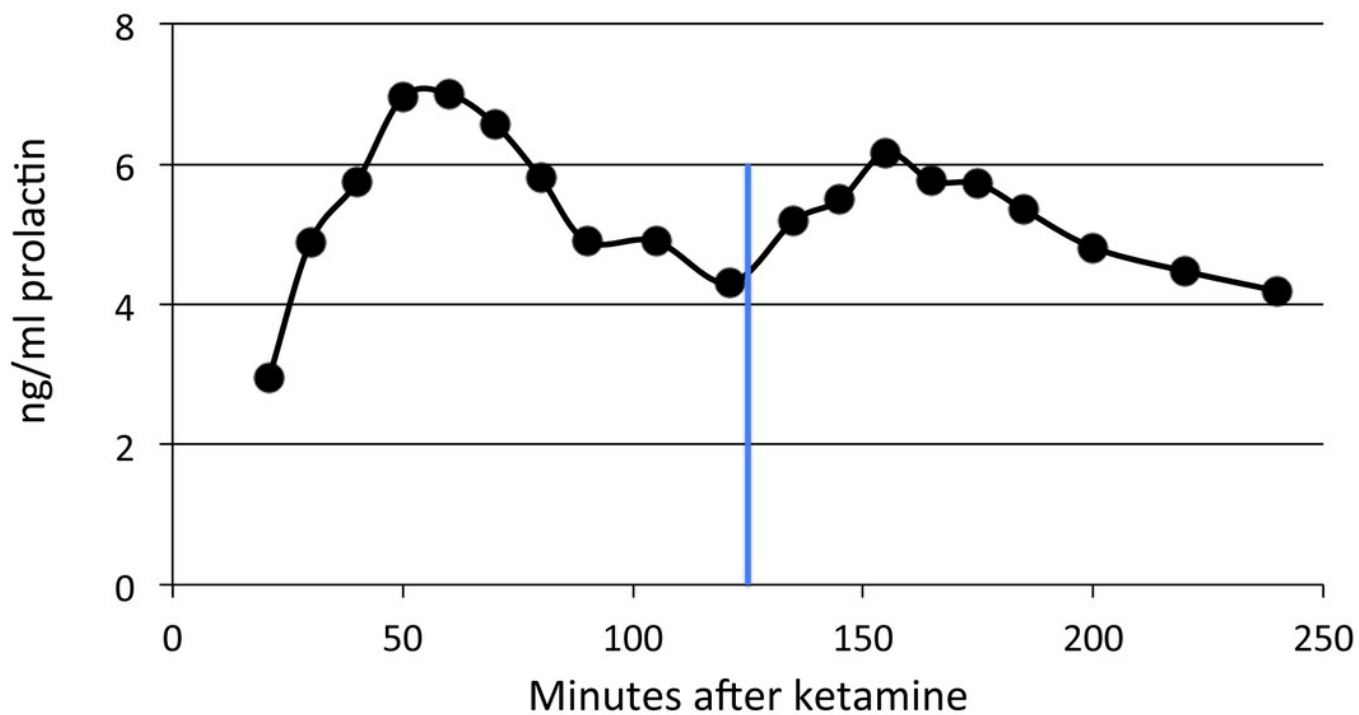


Figure 5

Time course of pramipexole effects on plasma prolactin

A single dose of 0.2 mg/kg of pramipexole (marked by the blue line) was given 180 minutes after ketamine.

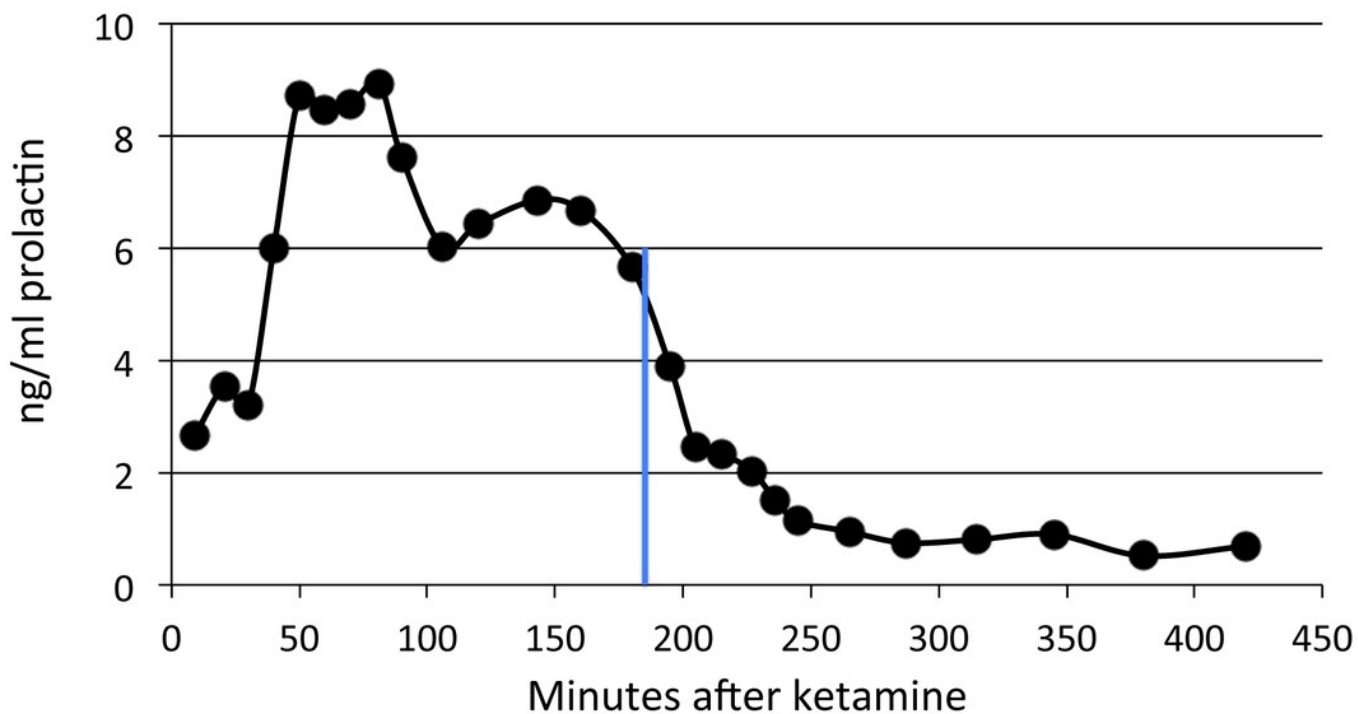


Figure 6

Dose-response curve for effect of SKF38393 on prolactin

Prolactin plasma concentration after SKF38393 is plotted as the difference from each day's pre-drug concentration. The inset shows a close-up of the data from the two juveniles.

Adults and juveniles differed in their responses (repeated measures ANOVA, main effect of age group $F_{1,3}=18.068$, $p=.024$; dose \times age group interaction $F_{7,21}=4.780$, $p=.077^*$; main effect of dose $F_{7,21}=5.154$, $p=.068^*$).

*=Greenhouse-Geisser correction for nonsphericity

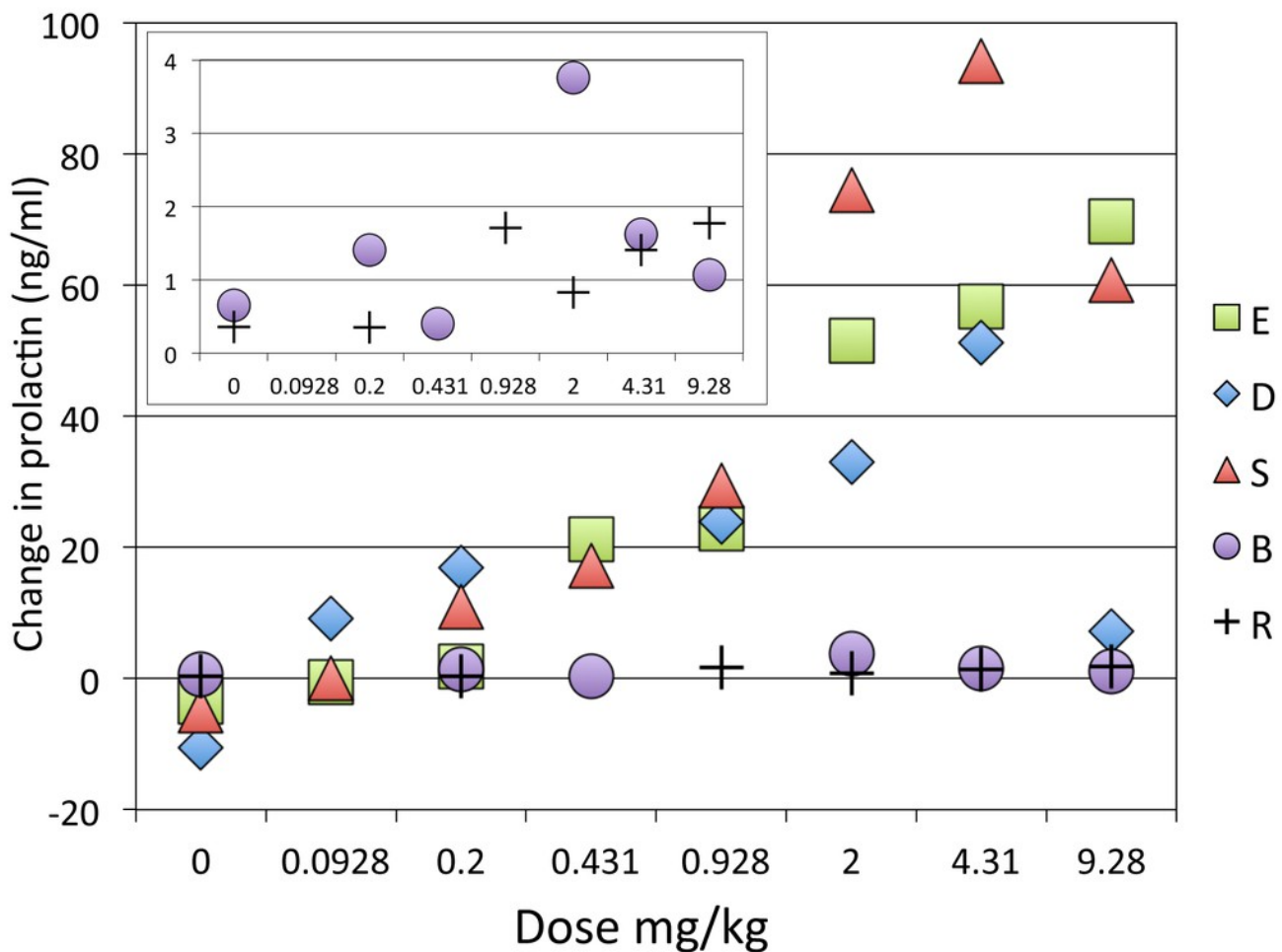


Figure 7

ED₅₀ for the mean prolactin response to SKF38393

The mean plasma [PRL] response to SKF38393 in the single-dose studies was plotted against log(dose). The response at each dose was defined as post-drug minus pre-drug [PRL] (hence the nonzero “response” in the absence of drug). A sigmoid E_{max} model was fit to the data from 0 to 4.31mg/kg as described in Materials & Methods under “Determination of ED₅₀.”

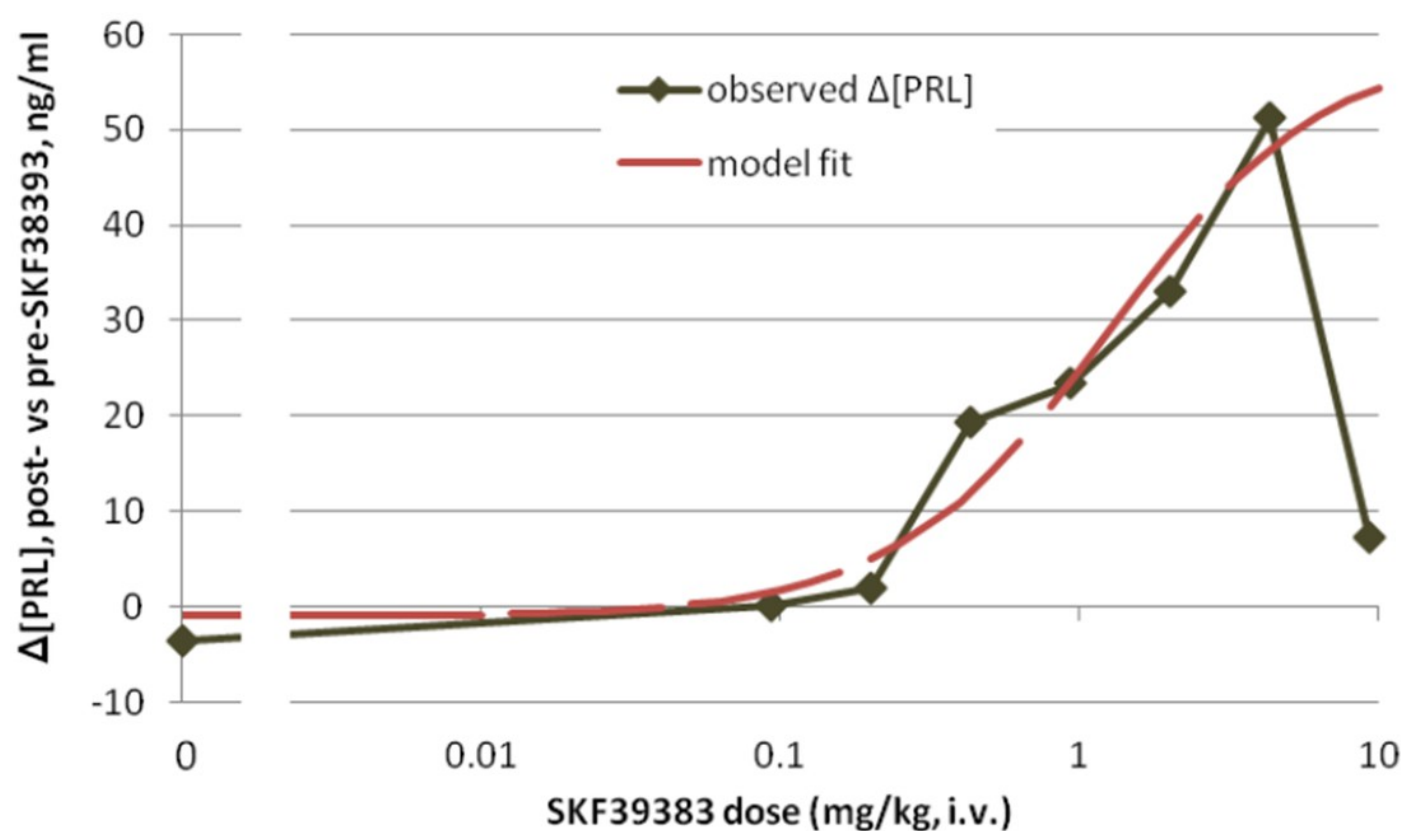
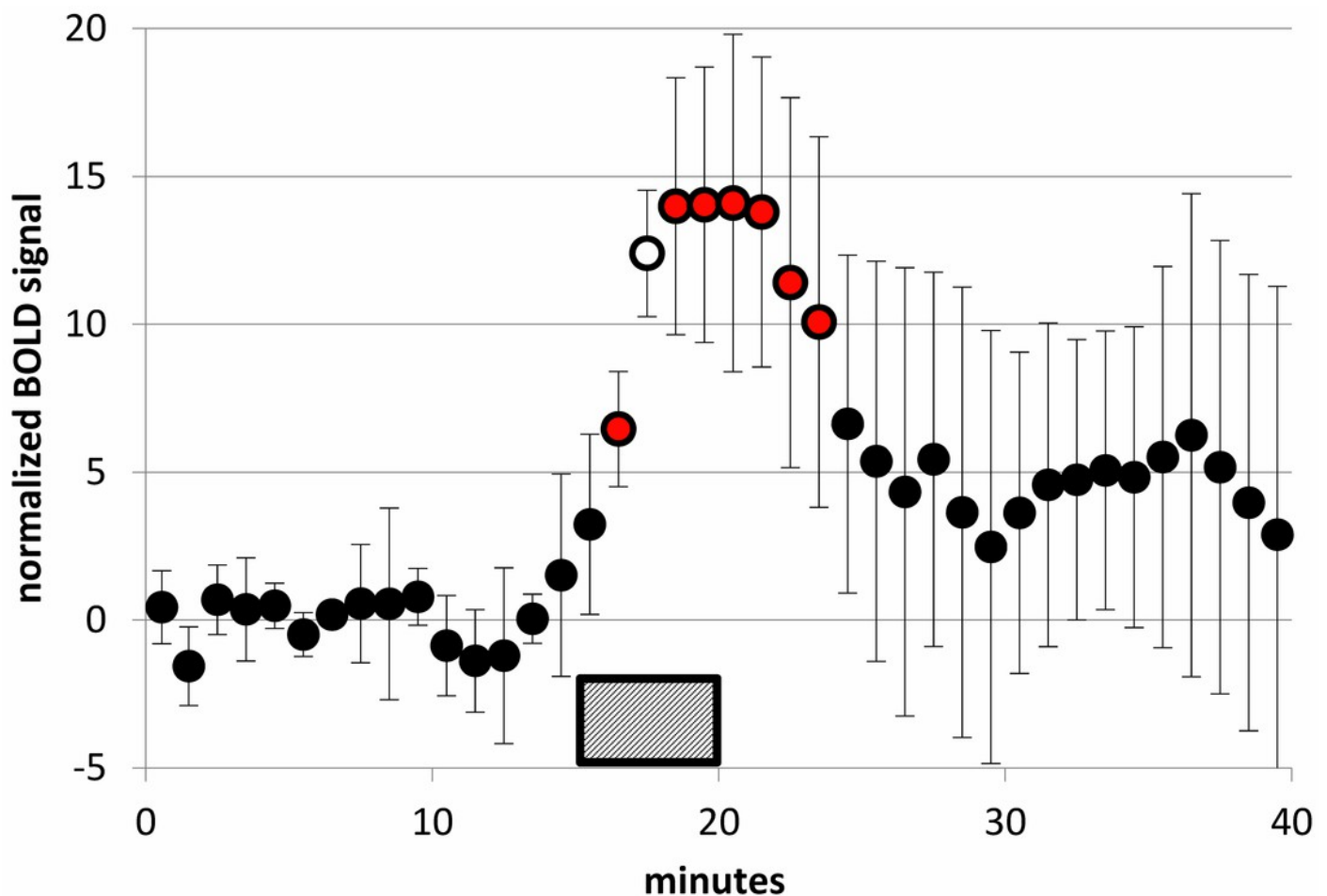


Figure 8

SKF38393 caused a clear BOLD signal response

BOLD signal in the midbrain VOI during a 40-minute functional MRI scan, after subtracting the linear trend computed from the first 15 minutes of data. Data plotted here are from each animal's 2mg/kg day from the SKF38393 single-dose studies (mean \pm S.D., N=5). SKF38393 2mg/kg was given i.v. over the time interval indicated (15-20 min). Markers filled with red indicate points that differ significantly from zero ($p < 0.05$, 2-tailed single-sample t test); the marker filled with white indicates $p = 0.0002$.



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Figure 10

Dose-response curves for the effect of SKF38393 on BOLD signal

The AUC_{15-30} at each dose of SKF38393 is plotted (mean, N=5) for midbrain, hypothalamus, pituitary, and striatum. AUC_{15-30} is defined in the last paragraph of Methods.

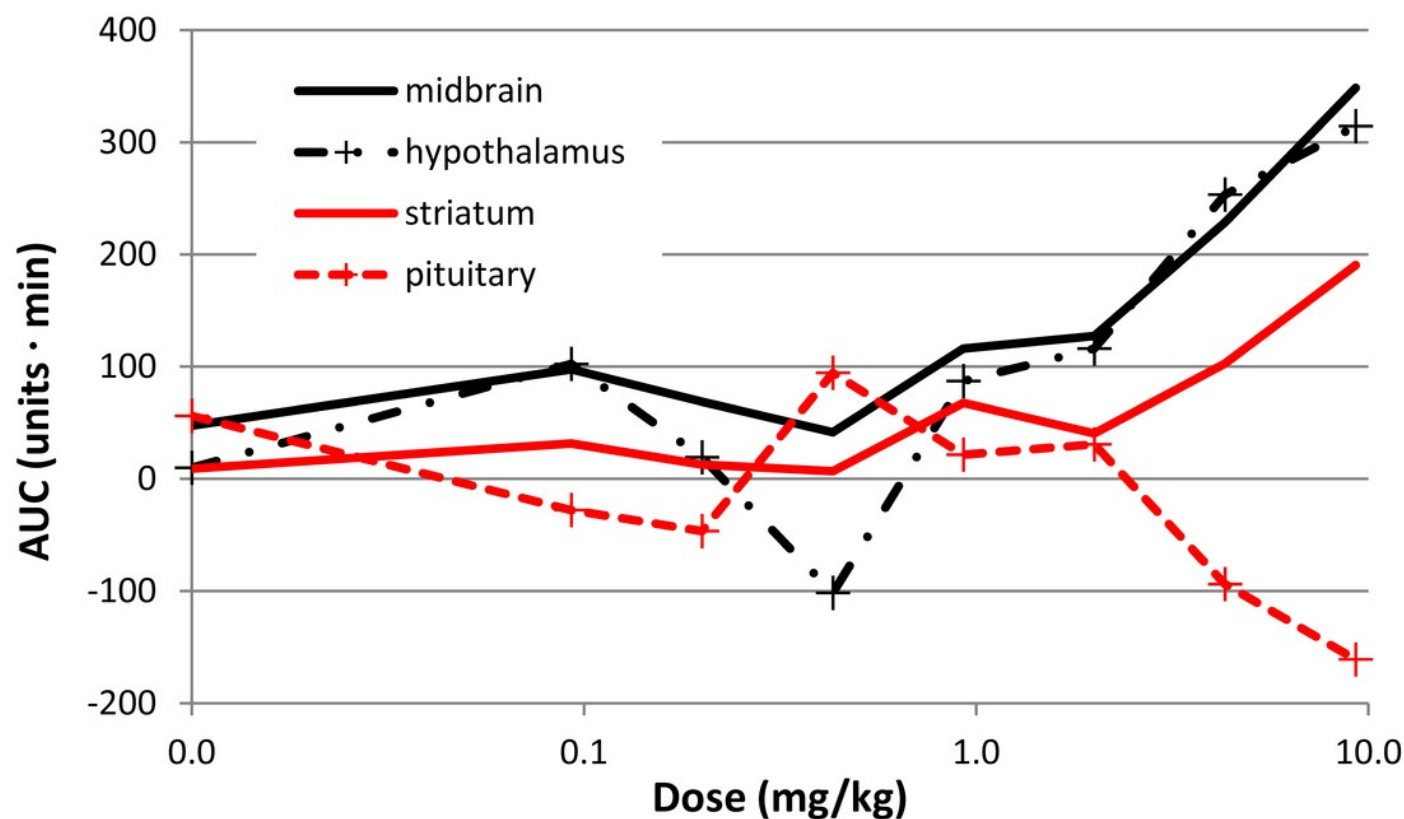


Table 1 (on next page)

Baseline prolactin plasma concentration, and peak increase in prolactin with anesthesia alone, by age

Subject	Age at start of study (years)	Baseline prolactin concentration (ng/ml), mean \pm S.D.	Increase in prolactin concentration with anesthesia
R	4.08	2.62 \pm 0.35	354 %
T	4.25	3.04 \pm 0.41	334 %
B	4.33	2.64 \pm 0.34	236 %
E	6.75	4.05 \pm 0.94	473 %
S	6.92	3.50 \pm 0.96	1198 %
D	14.08	23.5 \pm 6.02	410 %

Table 2_(on next page)

Responses to pramipexole by dose (N=1)

AUC₁₅₋₃₀ is defined in the last paragraph of Methods.

* = The prolactin response after the saline control is nonzero because it is defined as post-minus pre-drug, and prolactin levels are gradually declining following their initial post-ketamine peak (see Fig.1).

† = not available (marked artifact in numerous frames during this run)

Dose (mg/kg)	Change in prolactin plasma concentration (ng/ml)	BOLD signal response, midbrain (AUC₁₅₋₃₀)	BOLD signal response, pituitary (AUC₁₅₋₃₀)	BOLD signal response, hypothalamus (AUC₁₅₋₃₀)	BOLD signal response, striatum (AUC₁₅₋₃₀)
0	-0.62*	†	†	†	†
0.00928	-2.73	130.5	15.9	115.1	140.2
0.04310	-8.10	54.0	-92.4	-22.6	60.6
0.09280	-3.53	47.3	-80.2	121.2	73.3
0.20000	-6.04	187.0	-92.7	-85.2	54.2