Prolactin and fMRI response to SKF38393 in the baboon

Brad D. Miller, Lauren A. Marks, Jonathan Koller, Blake J. Newman, G. Larry Bretthorst, Kevin J Black

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 D_1-like agonists SKF82958 and SKF38393 or the D_2-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 ED_{50}(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_1receptor agonist SKF38393 produces clear plasma prolactin and phMRI dose-response curves.

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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.

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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; Perlmutter, 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 D_2 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). D_1 receptor activation also increases prolactin levels most likely by inhibiting the D_2 pathway (Curlewis et al 1993; Colthorpe et al 1998; Durham et al 1998; Freeman et al 2000).

SKF82958 is a full agonist at dopamine D_1-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 D_3-preferring D_2-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 D_1 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 D_1 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

65 minutes after parenteral administration in the rat or sheep (Cocchi et al 1987, Tanimoto et al
66 1987, Curlewis et al 1993), and prolactin levels return to baseline in about an hour. When given
67 orally, the delay is 60-150 minutes, returning to baseline within two to three hours (in humans;
68 Fabbrini et al 1988). Some, but not all, studies report dose-response characteristics with regard to
69 prolactin secretion (Salama 1986, Cocchi et al 1987, Tanimoto et al 1987, Fabbrini et al 1988,
70 Curlewis et al 1993, Saller and Durham et al 1998).

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

Materials & Methods

Subjects

77 The study was approved by the Washington University Animal Studies Committee (protocol
78 # 20090112). We studied six healthy male olive baboons (*Papio anubis*). This species was chosen
79 with a goal of later translation to human studies; specifically, baboon anatomy and
80 neuroendocrine responses are similar to humans', and their brain volume is big enough to allow
81 neuroimaging methods borrowed from human fMRI (Black et al 2009). Three were fully adult
82 (age 6.8-14 years) and three were juveniles (age 4 years). In captive male baboons, puberty
83 occurs at about 3.5 years of age (VandeBerg 2009, p. xxi), and the testes had descended in all
84 subjects before these studies began.

Overall approach

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

Time course studies

Anesthesia alone

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

With dopamine agonist

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

104   The dopamine agonist was given two to three hours after ketamine, because although prolactin
105   concentration was still elevated at these times as a consequence of the anesthetics, it was
106   significantly reduced from its peak at around 60 minutes and was starting to level off (see
107   Results: anesthesia alone studies). Blood samples were taken at various intervals before the
108   agonists and at 4- to 20-minute intervals afterward for a total of up to seven hours. These studies
109   involved either one dose of the dopamine agonist or three increasing doses, each separated by one
110   hour. The drug was infused i.v. over a five-minute period with a syringe pump (Harvard Model
111   44). Drug infusion time was reckoned from the end of the five-minute infusion period. Doses for
112   SKF38393 ranged from 0.2 to 5.0 mg/kg, SKF82958 was given at 0.1 mg/kg, and pramipexole
113   was given at 0.2 mg/kg.

114   **Single-dose dopamine agonist 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.

120   Five animals received varying doses of SKF38393, each dose on a different day. The order of
121   doses was random in each subject. The doses used were 0, 0.0928, 0.2, 0.431, 0.928, 2.0, 4.31,
122   and 9.28 mg/kg; not all subjects received all doses. A sixth animal received pramipexole at doses
123   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.

128   **Anesthesia**

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.

138   **Drug preparation**

139   SKF82958 (catalog # C130, Sigma-Aldrich, St Louis, MO) and SKF38393 (catalog # D047,
140   Sigma-Aldrich, St Louis, MO) were mixed fresh the day of the study, the pH was adjusted with
141   NaOH, then the solution was filter sterilized (0.22μm) and stored on ice in a light-tight container
142   until use. SKF82958 was prepared with sterile 0.9% saline at 1.0 mg/ml and the pH adjusted to
143   6.0-6.5. SKF38393 was prepared with sterile water from 1.0 to 7.3 mg/ml and the pH adjusted to
144   5.5-6.0. Pramipexole (a gift from Boehringer-Ingelheim Pharmaceuticals, Inc., Ridgefield, CT)
145   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.

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%.

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   192×192mm, 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   256×256mm, 192 slices, and TR 3200ms. Voxel size was approximately $(0.8mm)^3$ 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 384×384mm, 32
161   slices, voxel size $(2.5mm)^3$, 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.

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).

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    $Q = \log_{10}(ED_{50})$ 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.

Results

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.

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

259   sigmoid E_{max} model with ED_{50}s of: hypothalamus, 1.59mg/kg; midbrain, 2.25mg/kg; pituitary,
260   2.74mg/kg; and striatum, 7.70mg/kg.

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.

Discussion

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.

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 D_1 agonist SKF82958 was from an *in vitro*
301 study (Burris et al 1991). Based on its action on D_1 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 D_1 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, Fabbrini 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 D_2-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 ED_{50} for SKF38393 was 1.3mg/kg. It seems relevant that the VOI with the closest BOLD ED_{50} value to this was hypothalamus, with an ED_{50} of 1.59mg/kg.

Conclusions

In the baboon, the D_1 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 D_2-like agonist pramipexole produced a decline in prolactin levels, and the D_1 agonist SKF82958 did not affect prolactin release.

Acknowledgments

References

Aubert, M.L., Grumbach, M.M. and Kaplan, S.L. 1974. Heterologous radioimmunoassay for plasma human prolactin (hPRL): values in normal subjects, puberty, pregnancy and in pituitary disorders. Acta Endocrinologica, 77: 460-476.

Ben-Jonathan, N. and Hnasko. R. 2001. Dopamine as a prolactin (PRL) inhibitor. Endocrine Reviews, 22: 724– 763. DOI: 10.1210/er.22.6.724

Bergman, J., Spealman, RD, Madras BK, and Rosenzweig-Lipson, S. 1996. Agonist efficacy and the behavioral effects of dopamine D1 receptor ligands: drug interaction studies in squirrel monkeys. J Pharmacol Exp Ther., 276: 942-950.

Black, K.J., Gado, M.H. and Perlmutter, J.S. 1997. PET measurement of dopamine D_2 receptor-mediated changes in striatopallidal function. Journal of Neuroscience, 17(9): 3168–3177.

Black, K.J., Hershey, T., Gado, M.H. and Perlmutter, J.S. 2000. Dopamine D_1 agonist activates temporal lobe structures in primates. J. Neurophysiol. 84: 549-557.

Black, K.J., Hershey, T., Koller, J.M., Videen, T.O., Mintun, M.A., Price, J.L. and Perlmutter, J.L. 2002. A possible substrate for dopamine-related changes in mood and behavior: Prefrontal and limbic effects of a D_3-preferring dopamine agonist. Proc. Natl. Acad. Sci. U. S. A., 99: 17113–17118. DOI: 10.1073/pnas.012260599

Black, K.J., Hershey, T., Moerlein, S.M., Perlmutter, J.S. 2009. Neuroimaging in baboons. Pp. 327-350 *in* VandeBerg, J.L., WilliamsBlangero, S., Tardif, S.D. (eds.), **The Baboon in Biomedical Research**. New York: Springer. DOI: 10.1007/978-0-387-75991-3_17

Black KJ, Koller JM, Campbell MC, Gusnard DA, Bandak SI. 2010. Quantification of indirect
pathway inhibition by the adenosine A_{2a} antagonist SYN115 in Parkinson disease.
J Neurosci 30:16284-16292. DOI: 10.1523/JNEUROSCI.2590-10.2010

Black, K.J., Koller, J.M., Miller, B.D. 2013. Rapid quantitative pharmacodynamic imaging by a
novel method: Theory, simulation testing and proof of principle. PeerJ 2013; 1:e117. DOI:
10.7717/peerj.117

Black, K.J., Snyder, A.Z., Koller, J.M., Gado, M.H., Perlmutter, J.S. 2001. Template images for
nonhuman primate neuroimaging 1. Baboon. NeuroImage 2001; 14(3):736-743.
DOI: 10.1006/nimg.2001.0752

Bretthorst, G. L. 1988. **Bayesian Spectrum Analysis and Parameter Estimation**. Lecture Notes
in Statistics, Vol. 48 (series eds. Berger, J., Fienberg, S., Gani, J., Krickeberg, K., and
Singer, B.). New York: Springer-Verlag.

Bretthorst, G. L., Marutyan, K. 2012. Bayesian Analysis of Common NMR Problems, v. 4.0
(software). Available at http://bayesiananalysis.wustl.edu (accessed 08 May 2013).

Burris, T.P., Stringer LC, Freeman ME. 1991. Pharmacologic evidence that a D2 receptor subtype
mediates dopaminergic stimulation of prolactin secretion from the anterior pituitary gland.
Neuroendocrinology, 54: 175-183.

Chen YCI, Galpern WR, Brownell AL,Matthews RT, Bogdanov M, Isacson O, Keltner JR, Beal
MF, Rosen BR, Jenkins BG. 1997. Detection of dopaminergic neurotransmitter activity
using pharmacologic MRI: correlation with PET, microdialysis, and behavioral data.
Magnetic Resonance in Medicine 38:389–398 DOI 10.1002/mrm.1910380306.

Cocchi, D., Ingrassia, S., Rusconi, L., Villa, I. and Muller, E.E. 1987. Absence of D1 dopmaine
receptors that stimulate prolactin release in the rat pituitary. Eur. J. Pharmacology, 142:
425-429.

Colthorpe, K.L., Anderson, S.T., Martin, G.B. and Curlewis, J.D. 1998. Hypothalamic dopamine
D1 receptors are involved in the stimulation of prolactin secretion by high environmental
temperature on the female sheep. Journal of Neuroendocrinology, 10: 503-509.

Crozier, T.A., Müller, J.E., Quittkat, D., Sydow, M., Wuttke, W. and Kettler, D. 1994. Effect of
anaesthesia on the cytokine responses to abdominal surgery. Br. J. Anesthesia, 72: 280-
285.

Curlewis, J.D., Clarke, I.J. and McNeilly, A.S. 1993. Dopamine D1 receptor analogues act
centrally to stimulate prolactin secretion in ewes. Journal of Endocrinology 137: 457-464.

Davis, R. and Huffman, R. D. 1968. **A Stereotaxic Atlas of the Brain of the Baboon**. Austin,
Texas: University of Texas Press.

Dixon, A.L. Prior, M., Shah, Y.B., Morris, P.G., Young, A. and Joseph, M. 2001. Effect of
dopamine D1 agonist and antagonist on the rat brain as determined using BOLD fMRI.
Proc. Intl. Soc. Mag. Reson. Med. 9: 1340.

Durham, R.A., Johnson, J.D., Eaton, M.J., Moore, K.E. and Lookingland, K.J. 1998 Opposing
roles for dopamine D1 and D2 receptors in the regulation of hypothalamic
tuberoinfundibular dopamine neurons. European Journal of Pharmacology , 355: 141-147.

Ehara, Y., Yen, S. S. C. and Siler, T. M. 1975. Serum prolactin levels during puberty. Am. J.
Obstet. Gynaecol., 121: 995-997.

Engber, T.M., Anderson, J.J., Boldry, R.C., Kuo, S. and Chase, T.N. 1993. N-methyl-d-aspartate
receptor blockade differentially modifies regional cerebral metabolic responses to D1 and
D2 dopamine agonists in rats with a unilateral 6-hydroxydopamine lesion. Neuroscience,
54: 1051-1061.

Fabbrini, G., Braun, A., Mouradian, M. M., Tamminga, C. A. and Chase, T. N. 1988. Dopamine
D-1 receptor agonist stimulation of prolactin secretion in man. J Neural Transm, 71: 159-
163.

429 Finkelstein, J.W., Kapen, S., Weitzman, E.D., Hellman, L and Boyar, R.M. 1978. Twenty-four-
430   hour plasma prolactin patterns in prepubertal and adolescent boys. Journal of Clinical
431   Endocrinology & Metabolism. 47: 1123-1128.
432 Freeman, M.E., Kanyicska, B., Lerant, A. and Nagy, G. 2000. Prolactin: structure, function, and
433   regulation of secretion. Physiol. Rev., 80: 1523-1631.
434 Gilmore, J.H., Watts, V.J., Lawler, .CP., Noll, E.P., Nichols, D.E. and Mailman, R.B. 1995. "Full"
435   dopamine D1 agonists in human caudate: biochemical properties and therapeutic
436   implications. Neuropharmacology, 34: 481-488.
437 Guyda, H. J. and Friesen, H. G. 1973. Serum prolactin levels in humans from birth to adult life.
438   Pediatric Research, 7: 534-540.
439 Hayashi, T. and Tadokoro, S. 1984. Parallelism between avoidance-suppressing and prolactin-
440   increasing effects of antipsychotic drugs in rats. Japan. J. Pharmacol. 35: 451-456.
441 Hergovich, N., Singer, E, Agneter, E., Eichler, H.G., Graselli, U., Simhandl, C., and Jilma, B.
442   2001. Comparison of the effects of ketamine and memantine on prolactin and cortisol
443   release in men: A randomized, double-blind, placebo-controlled trial.
444   Neuropsychopharmacology, 24: 590–593. DOI: 10.1016/S0893-133X(00)00194-9
445 Herscovitch P. 2001. Can [15O]water be used to evaluate drugs? Journal of Clinical
446   Pharmacology 41:11S–20S. DOI 10.1177/009127001773744116.
447 Hershey T, Black KJ, Carl JL,McGee-Minnich L, Snyder AZ, Perlmutter JS. 2003. Long term
448   treatment and disease severity change brain responses to levodopa in Parkinson's disease.
449   Journal of Neurology, Neurosurgery, and Psychiatry 74:844–851
450   DOI 10.1136/jnnp.74.7.844.
451 Hershey T, Black KJ, Carl JL, Perlmutter JS. 2000. Dopa-induced blood flow responses in non-
452   human primates. Experimental Neurology 166:342–349 DOI 10.1006/exnr.2000.7522
453 Holford, N. H. G., and Sheiner, L. B. 1982. Kinetics of pharmacologic response. Pharmacol Ther
454   16: 143-166.
455 Hood, S.D., Potokar, J.P., Davies, S.J.C., DA Hince, D.A., Morris, K., Seddon, K.M., Nutt, D.J.
456   and Argyropoulos, S.V. 2010. Dopaminergic challenges in social anxiety disorder:
457   evidence for dopamine D3 desensitisation following successful treatment with
458   serotonergic antidepressants. J. Psychopharmacol., 24: 709-716.
459   DOI: 10.1177/0269881108098144
460 Jaber, M., Robinson, S. W., Missale, C. and Caron, M.G. 1996. Dopamine receptors and brain
461   function, Neuropharmacology, 35; 1503-1519.
462 Kondo, M., Udono T, Jin W, Shimizu K, Funakoshi M, Itoh M, Watanabe G, Groome NP, and
463   Taya K. 2000. Changes in plasma concentrations of inhibin A and inhibin B throughout
464   sexual maturation in the male chimpanzee. Endocr. J. 47: 707-714.
465   DOI: 10.1507/endocrj.47.707
466 Leblanc, H., Lachelin, G.C., Abu-Fadil, S. and Yen, S.S. 1976. Effects of dopamine infusion on
467   pituitary hormone secretion in humans. Journal of Clinical Endocrinology & Metabolism,
468   43: 668-674.
469 Lee, P.A., Jaffe, R.B. and Midgley, A.R. 1974. Serum gonadotropin, testosterone and prolactin
470   concentrations throughout puberty in boys: a longitudinal study. J. Clin. Endocrin. Metab.,
471   39: 664-672.
472 Marana, E., Annetta, M.G., Meo, F., Parpaglion, R., Galeone, M., Maussier, M.L and Marana,
473   R. 2003. Sevoflurane improves the neuroendocrine stress response during laparoscopic
474   pelvic surgery. Can. J. Anesth., 50: 348-354. DOI: 10.1007/BF03021031
475 Mierau, J. 1995. Pramipexole: a dopamine-receptor agonist for treatment of Parkinson's disease.
476   Clin. Neuropharmacol. 18: S195–S206.
477 Mierau, J., Schneider, F. J., Ensinger, H. A., Hio, C. L., Lajiness, M. E. and Huff, R. M. 1995.

Pramipexole binding and activation of cloned and expressed dopamine D2, D3 and D4 receptors. Eur. J. Pharmacol. 290: 29–36.

Morelli, M., Pontieri, F.E., Linfante, I., Orzi, F. and Di Chiara, G. 1993. Local cerebral glucose utilization after D1 receptor stimulation in 6-OHDA lesioned rats: effect of sensitization (priming) with a dopaminergic agonist. Synapse, 13: 264-269.

Norman, R.L., Quadri, S.K. and Spies, H.G. 1980. Differential sensitivity of prolactin release to dopamine and thyrotrophin-releasing hormone in intact and pituitary stalk-sectioned rhesus monkeys. Journal of Endocrinology, 84: 479-487.

Perlmutter JS, Rowe CC, Lich LL. 1993. In vivo pharmacological activation of dopaminergic pathways in primates studied with PET. Journal of Cerebral Blood Flow and Metabolism 13:S286.

Phillips-Conroy, J. E., Jolly, C. J., Rogers, J., and Ziegler, T. 2013. Serum prolactin and social behavior of Kindas and other baboons (poster abstract). American Journal of Physical Anthropology 150 (Suppl 56):221. Available at http://meeting.physanth.org/program/2013/session18/phillips-conroy-2013-serum-prolactin-and-social-behavior-of-kindas-and-other-baboons.html

Piercey, M. F. 1998. Pharmacology of pramipexole, a dopamine D3-preferring agonist useful in treating Parkinson's disease. Clin. Neuropharmacol. 21: 141–151.

Porta, F., Mussa, A., Concolino, D., Spada, M. and Ponzone, A. 2009. Dopamine agonists in 6-pyruvoyl tetrahydropterin synthase deficiency. Neurology. 73: 633-637. DOI: 10.1212/WNL.0b013e3181b38983

Puri, C.P., Puri, V and Anand Kumar, T.C. 1981. Serum levels of testosterone, cortisol, prolactin and bioactive luteinizing hormone in adult male rhesus monkeys following cage-restraint or anaesthetizing with ketamine hydrochloride. Acta Endocrinologica, 97: 118-124.

Quadri SK, Pierson C, Spies HG. 1978. Effects of centrally acting drugs on serum prolactin levels in rhesus monkeys. Neuroendocrinology 27:136-147.

Reber, A., Huber, P.R., Ummenhofer, W., Gürtler, C.M., Zurschmiede, C., Drewe, J., and Schneider, M. 1998. General anaesthesia for surgery can influence circulating melatonin during daylight hours. Acta Anaesthesiol Scand., 42: 1050-1056.

Rizvi, S.S.R., Altaf, S. Naseem, A.A., Asif, M., Rasul, Z. and Qayyum, M. 2001. The effect of ketamine hydrochloride anesthesia on basal and N-methyl-D,L-aspartate induced plasma prolactin secretion in the adult male rhesus monkey. Life Sci., 68: 1083–1093. DOI: 10.1016/S0024-3205(00)01010-9

Saller, C. F. and Salama, A. I. 1986. D-1 dopamine receptor stimulation elevates plasma prolactin levels. Eur J Pharmacol., 122: 139-142.

Samuels, E.R., Hou, R.H., Langley, R.W., Szabadi, E. and Bradshaw, C. M. 2006. Comparison of pramipexole and amisulpride on alertness, autonomic and endocrine functions in healthy volunteers. Psychopharmacology, 187: 498–510. DOI: 10.1007/s00213-006-0443-y

Schettini, G., Mastronardi, P., Scanni, E., Pinto, M., Forgione, A., Florio, T., Savastano, S., Merola, B., Lombardi, G., and Mazzarella, B. 1989. Antiemetic effect of the levo isomer of sulpiride (L-sulpiride) in humans. Minerva Anestesiol. 55: 239-243.

Schilling, J.C., Adamus, W.S. and Palluk, R. 1992 Neuroendocrine and side effect profile of pramipexole, a new dopamine receptor agonist, in humans. Clin Pharmacol Ther, 51: 541-548.

Tanimoto, K., Tamminga, C.A. and Chase, T.N. 1987. Elevation of plasma prolactin concentrations by intravenous SCH 23390 and SKF 38393 in conscious rats. Eur J Pharmacol., 144: 147-151.

Trugman JM and James CL. 1992. Rapid development of dopaminergic supersensitivity in reserpine-treated rats demonstrated with ^{14}C-2-deoxyglucose autoradiography. Journal of

527   Neuroscience 12:2875–2879.

528 Trugman, J.M. and James C.L. 1993. D1 dopamine agonist and antagonist effects on regional
529   cerebral glucose utilization in rats with intact dopaminergic innervation. Brain Res., 607:
530   270–274.

531 Trugman JM,Wooten GF. 1986. The effects of L-DOPA on regional cerebral glucose utilization in
532   rats with unilateral lesions of the substantia nigra. Brain Research 379:264–274
533   DOI 10.1016/0006-8993(86)90780-8.

534 VandeBerg JL. 2009. Introduction. Pp. xvii-xxiii *in* VandeBerg JL, Williams-Blangero S, Tardif
535   SD (eds.), **The Baboon in Biomedical Research**. New York: Springer.

536 Walker ML. 1987. Effects of anesthetic agents on the adrenocortical system of female baboons.
537   1987. Am J Primatol 13:325-332.

538 Wickings, E.J. and Nieschlag, E. 1980. Pituitary response to LRH and TRH stimulation and
539   peripheral steroid hormone in conscious and anaesthetized adult male rhesus monkeys
540   (*Macaca mulatta*). Acta Endocrinologica, 93: 287-293.

541 Zhang Z, Andersen AH, Avison MJ, Gerhardt GA, Gash DM. 2000. Functional MRI of
542   apomorphine activation of the basal ganglia in awake rhesus monkeys. Brain Research
543   852:290–296 DOI 10.1016/S0006-8993(99)02243-X.

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.
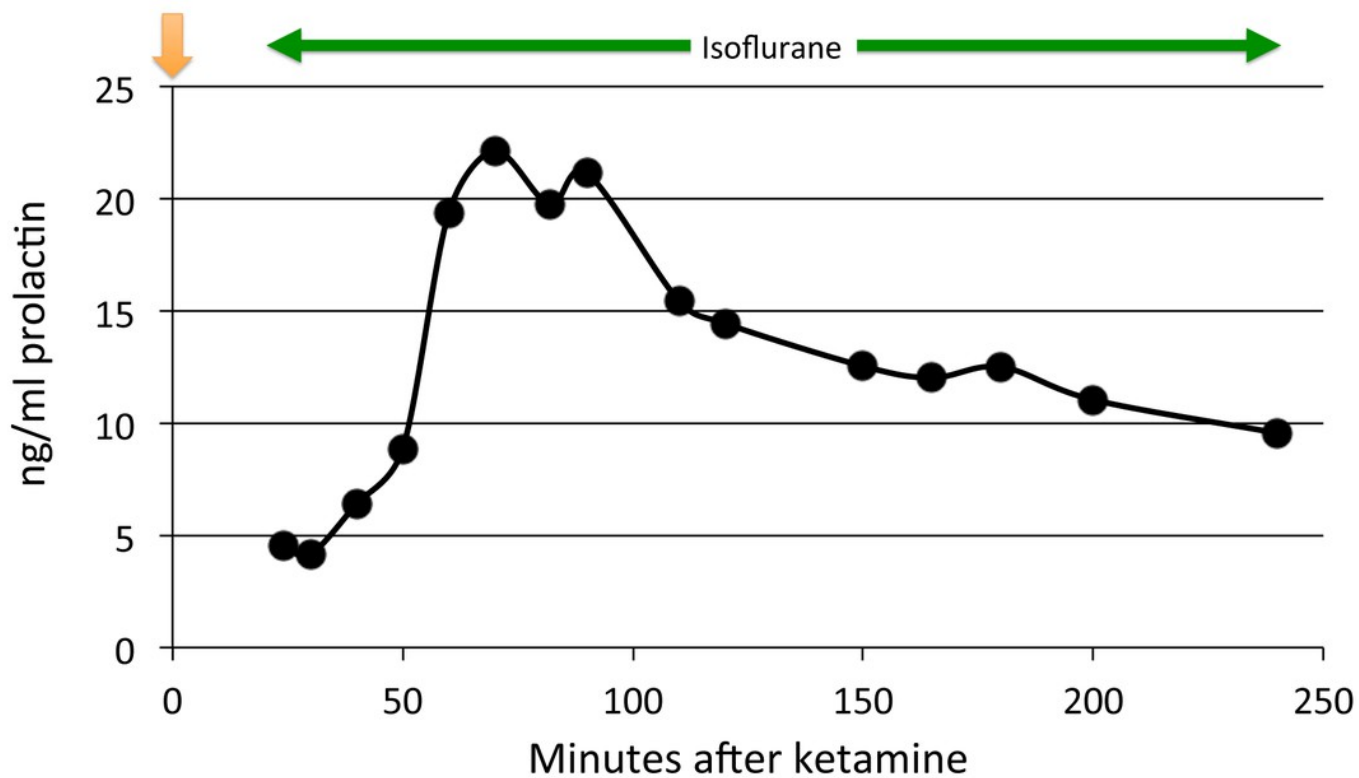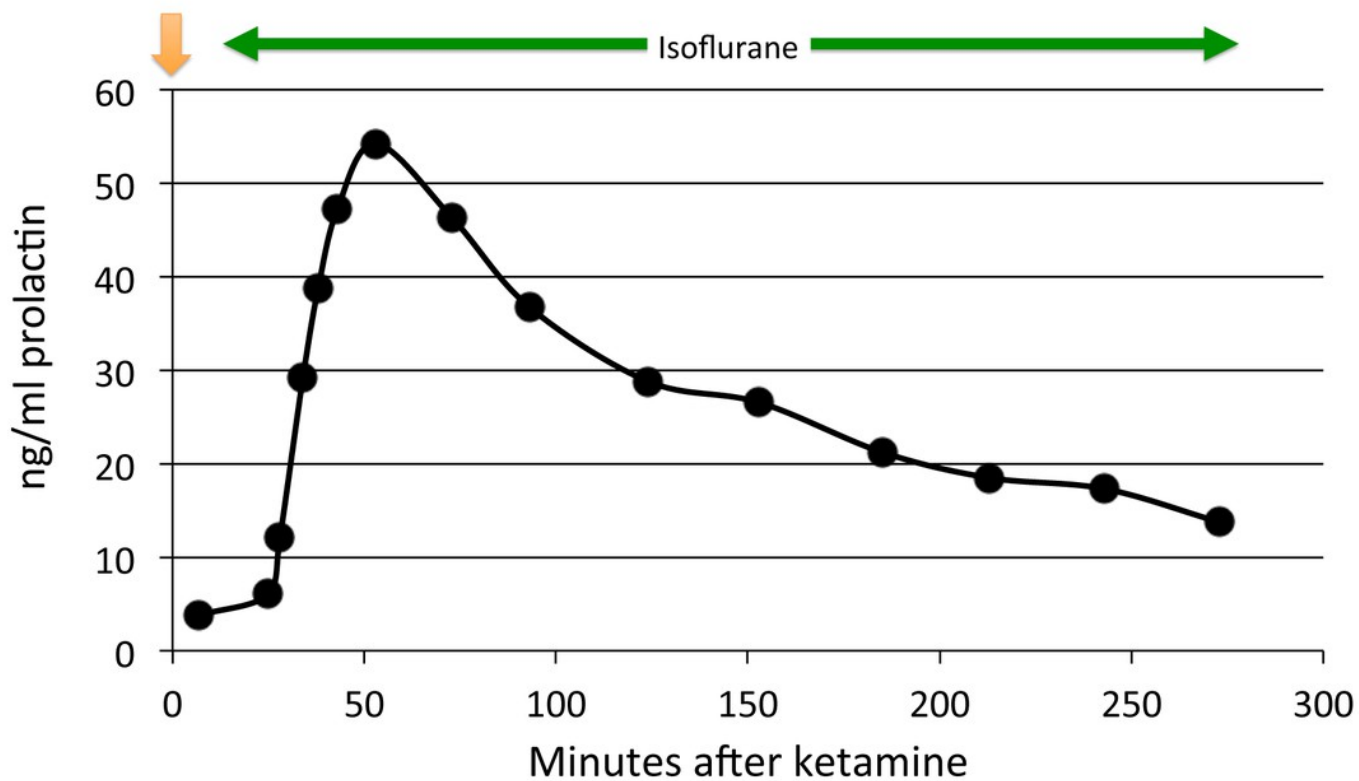
Figure 2

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 × 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
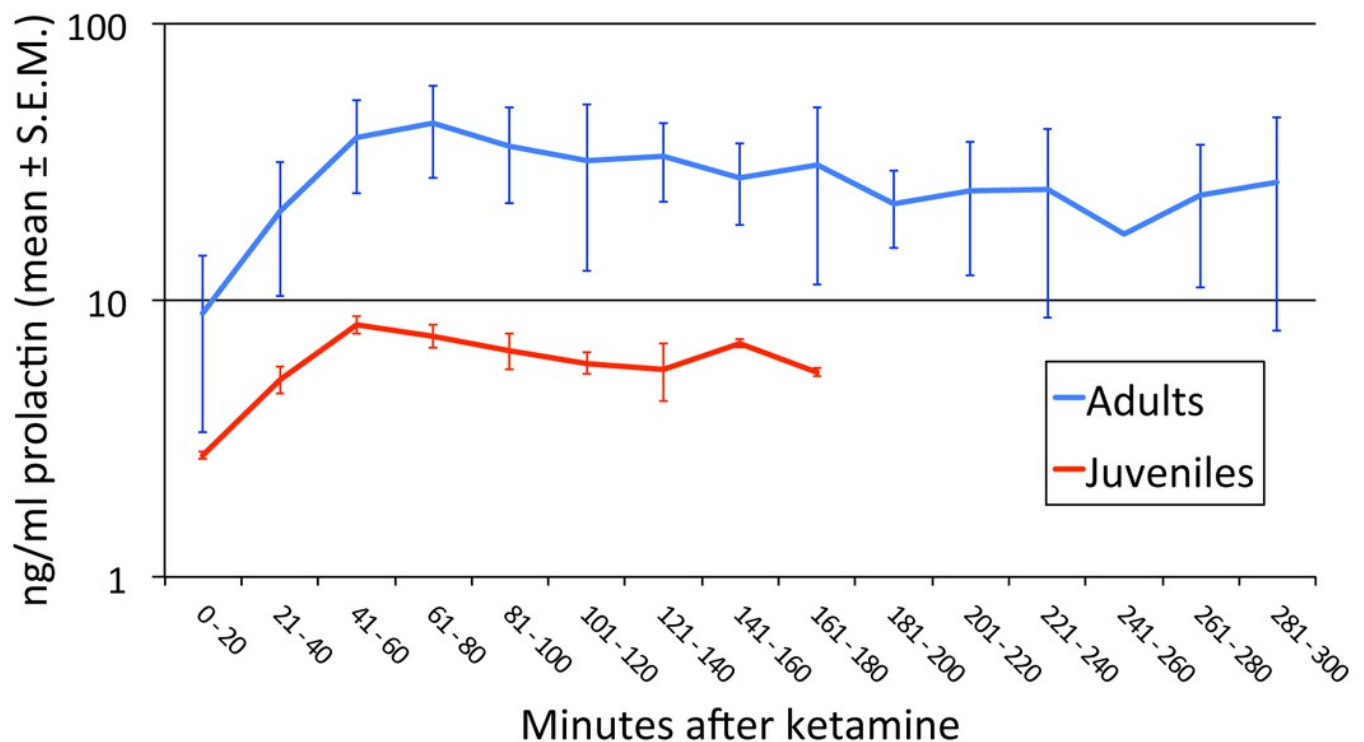
Figure 3

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).
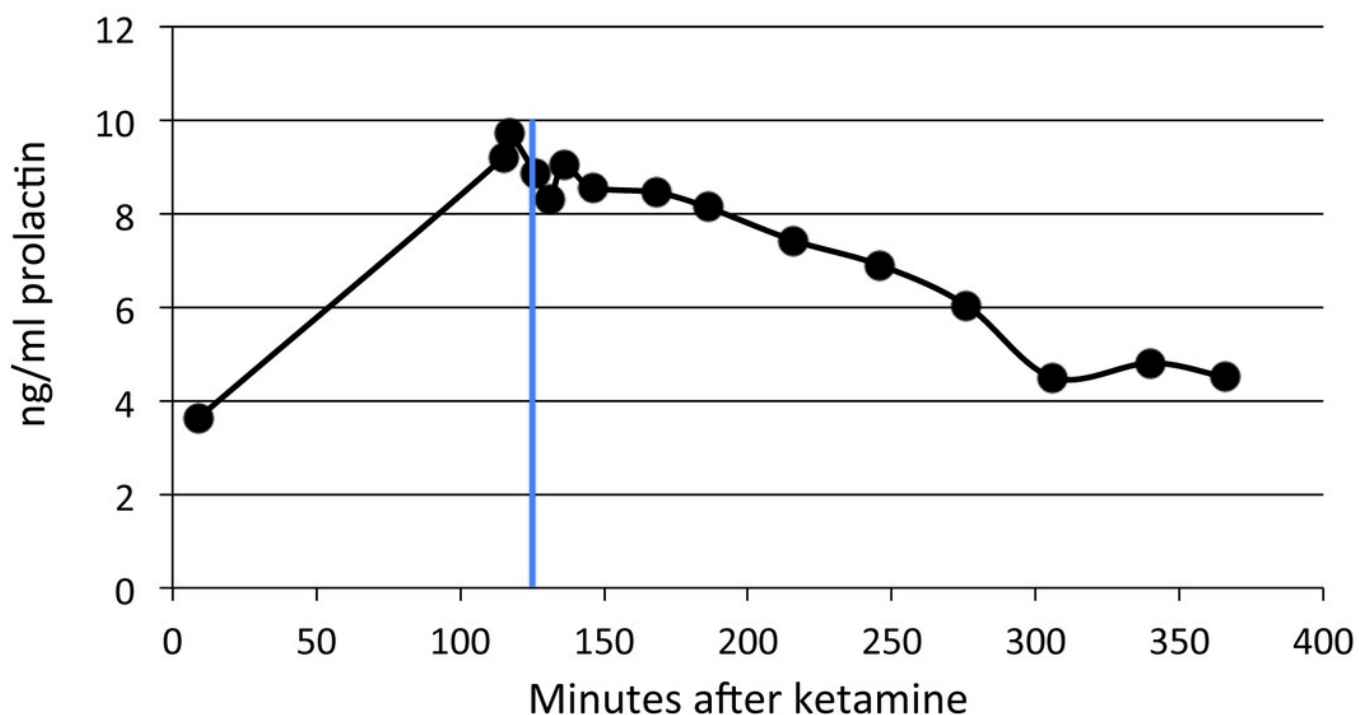
Figure 4

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).
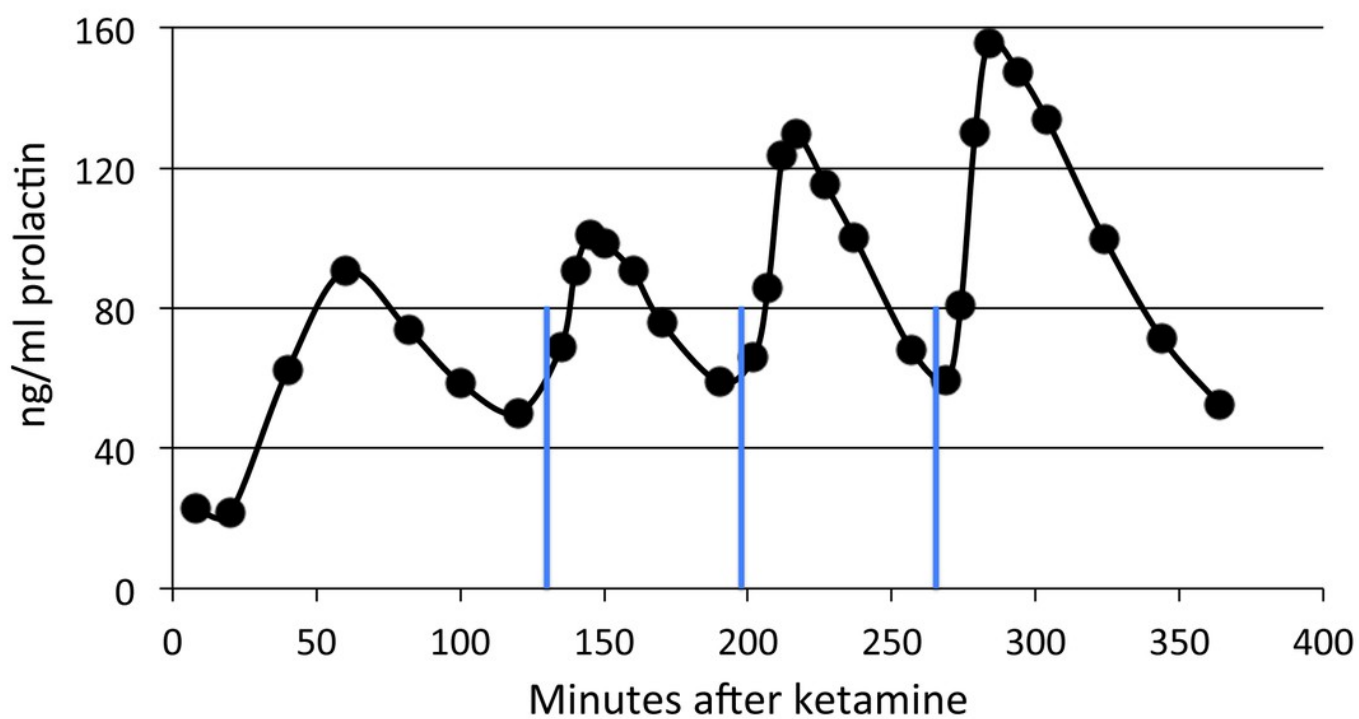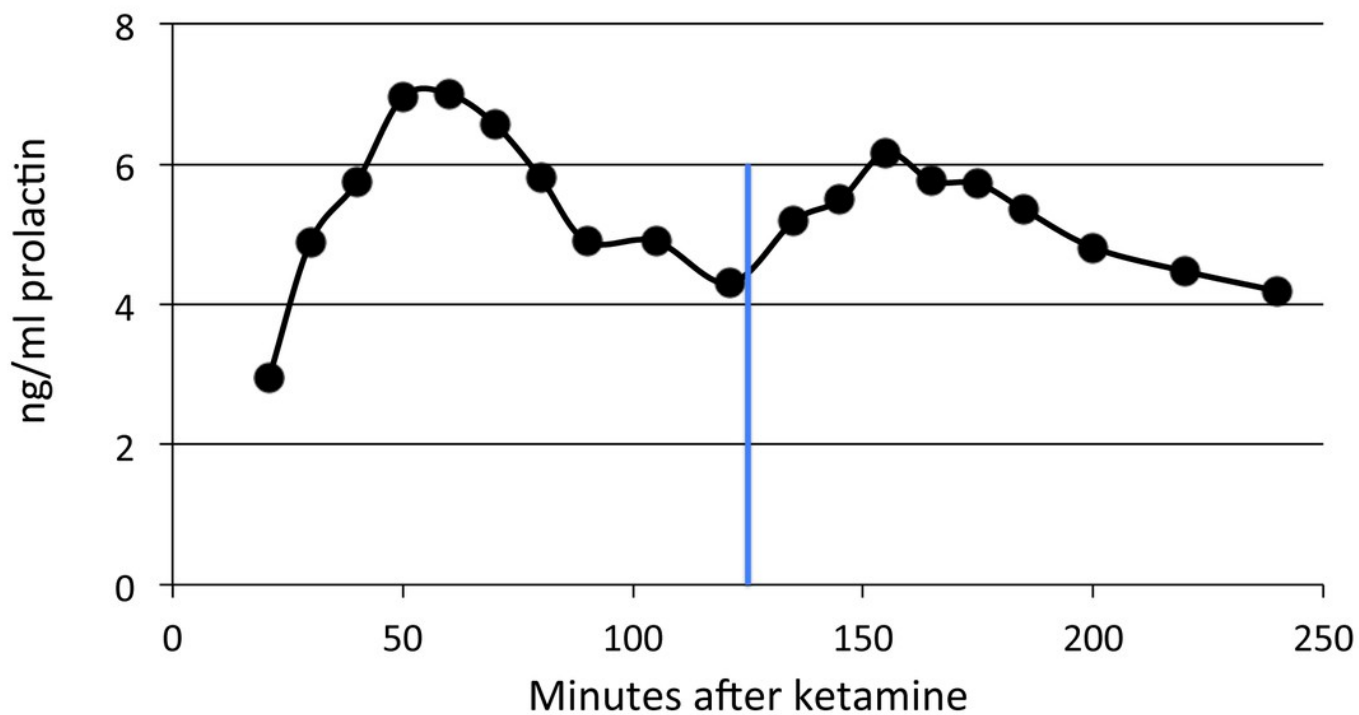
Figure 5

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.
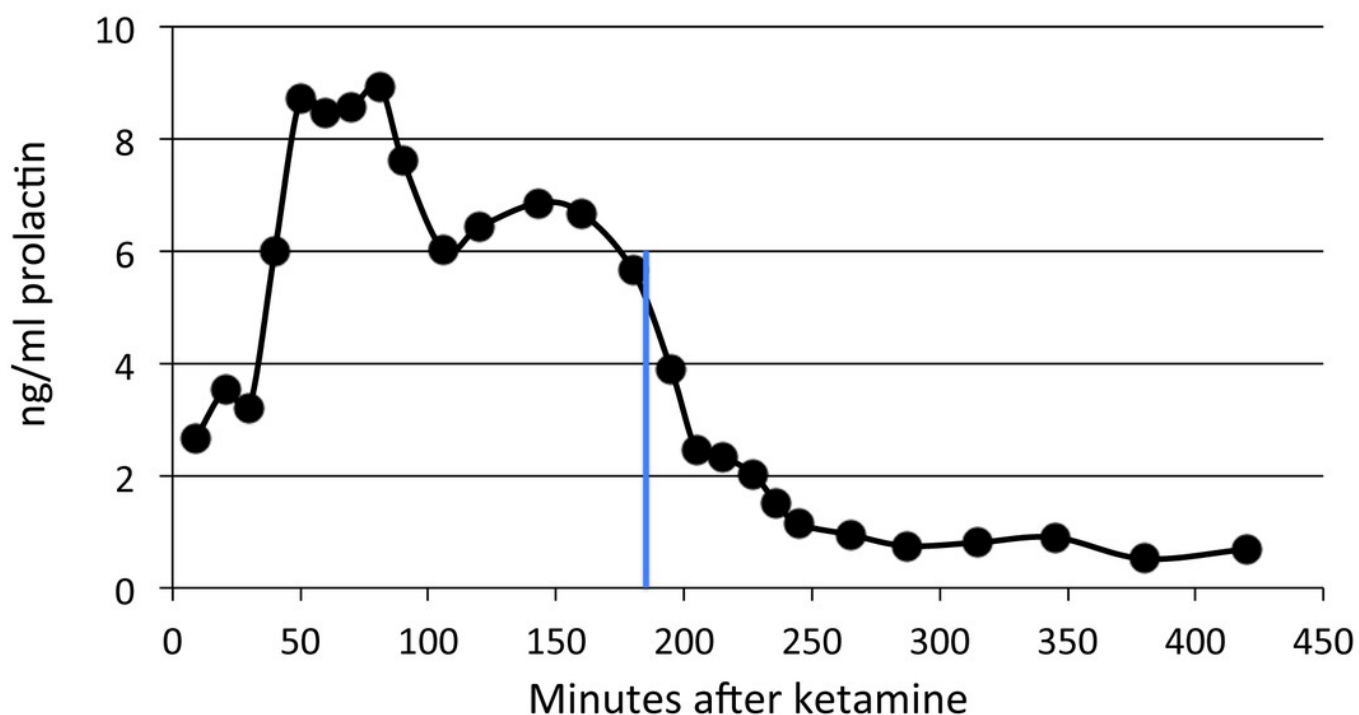
Figure 6

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 × age group interaction $F_{7,21}=4.780$, p=.077*; main effect of dose $F_{7,21}=5.154$, p=.068*).
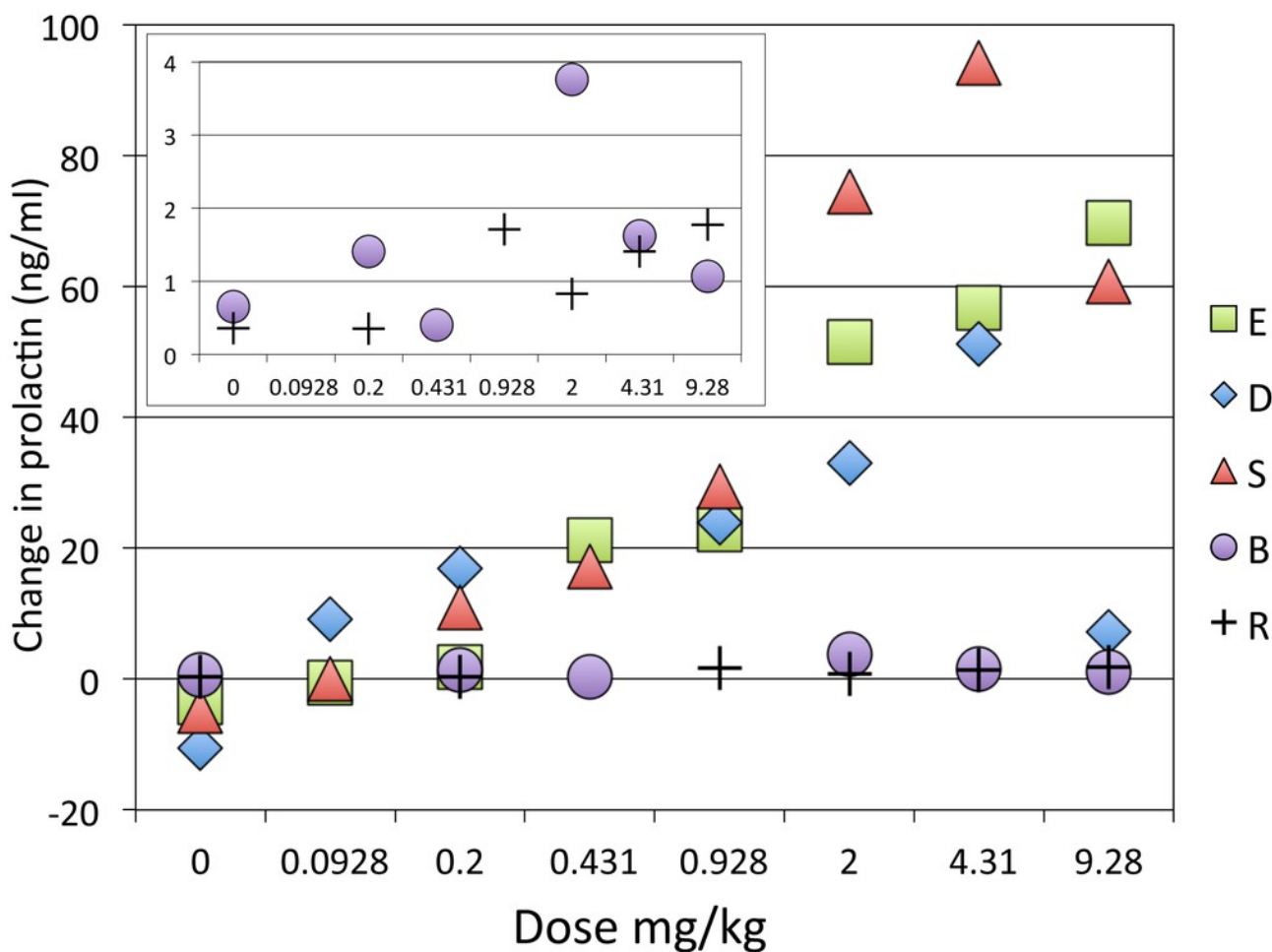
*=Greenhouse-Geisser correction for nonsphericity

Figure 7

ED_{50} 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 ED_{50}."
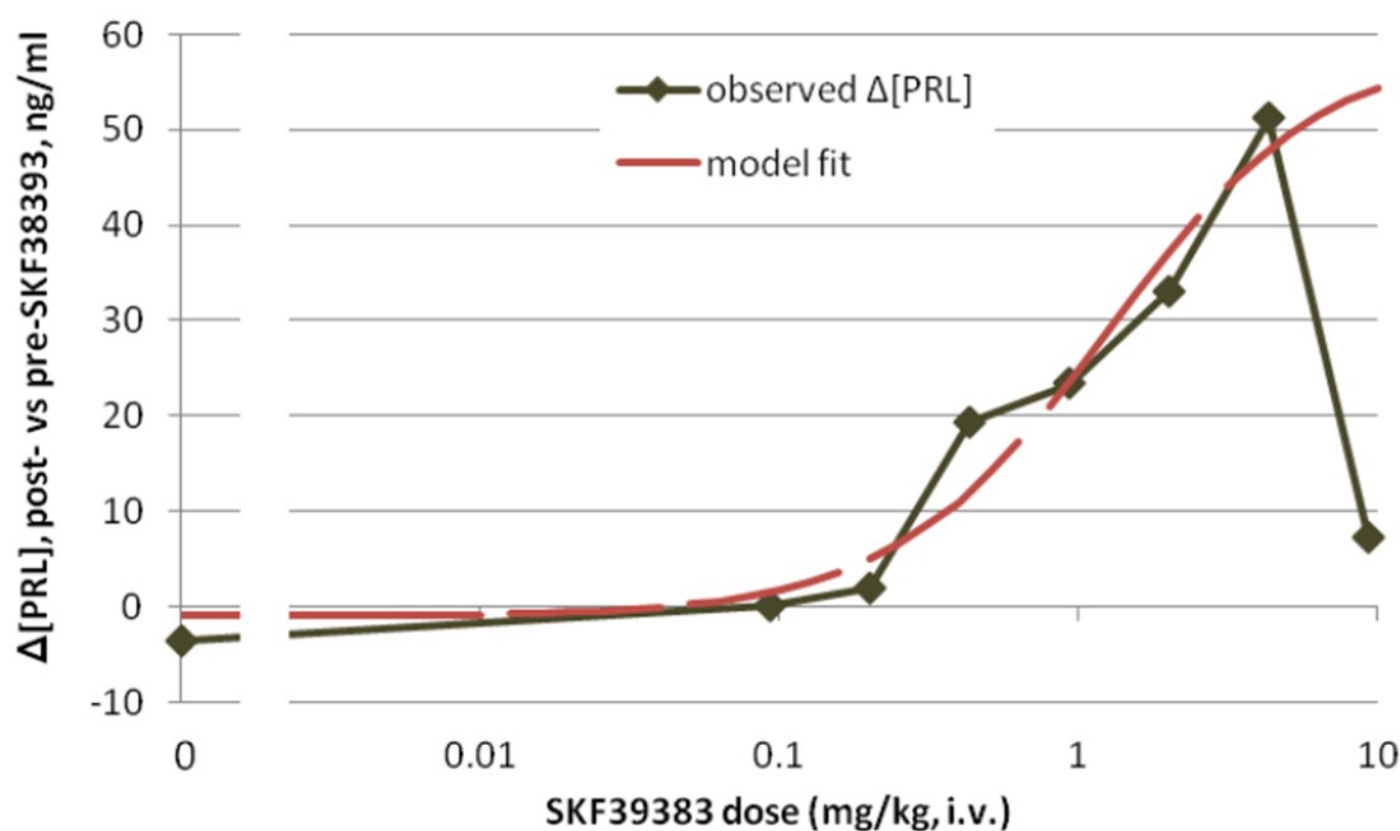
Figure 8

SKF38393 caused a clear BOLD signal response

BOLD signal 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 ± 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.
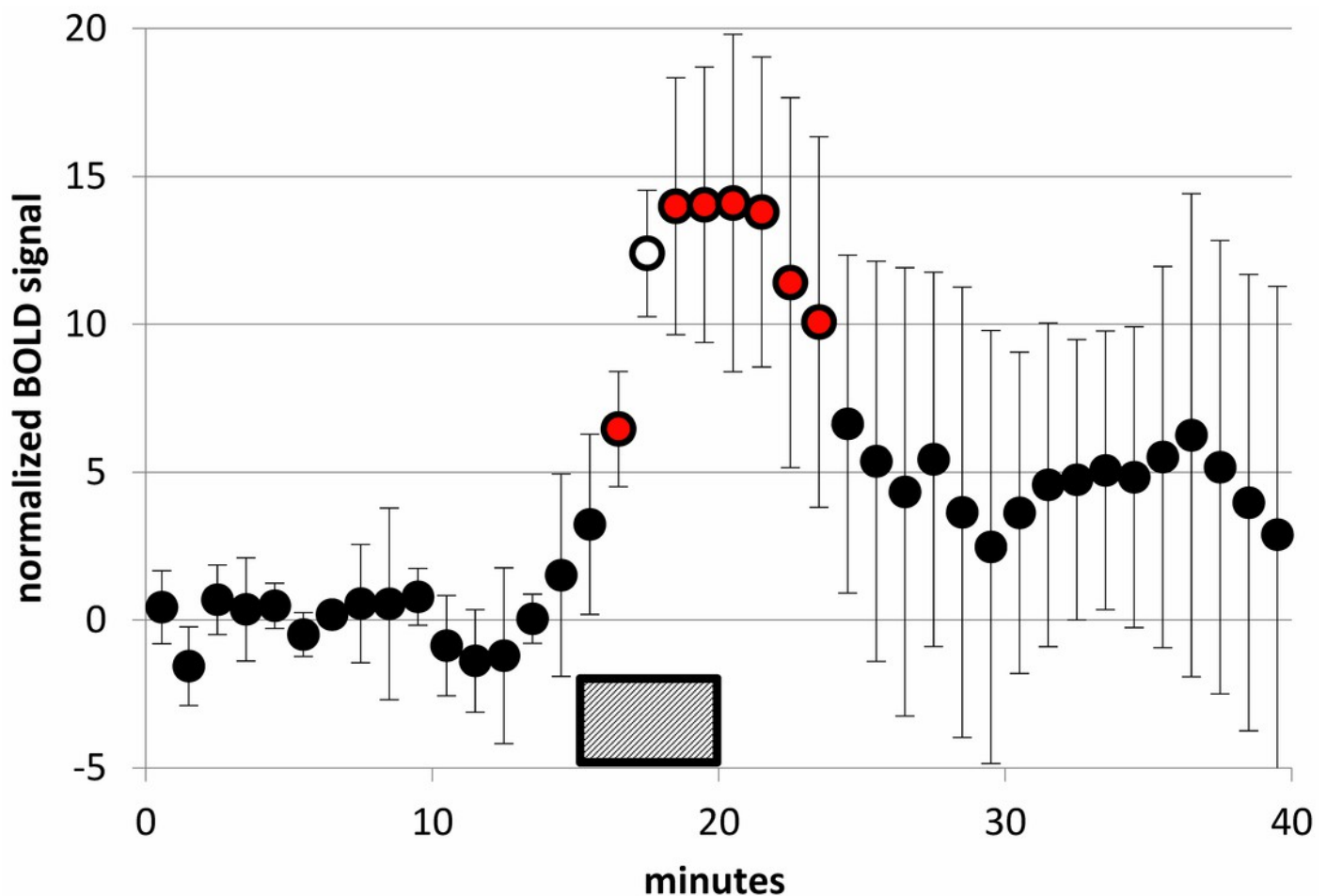
Figure 9

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.
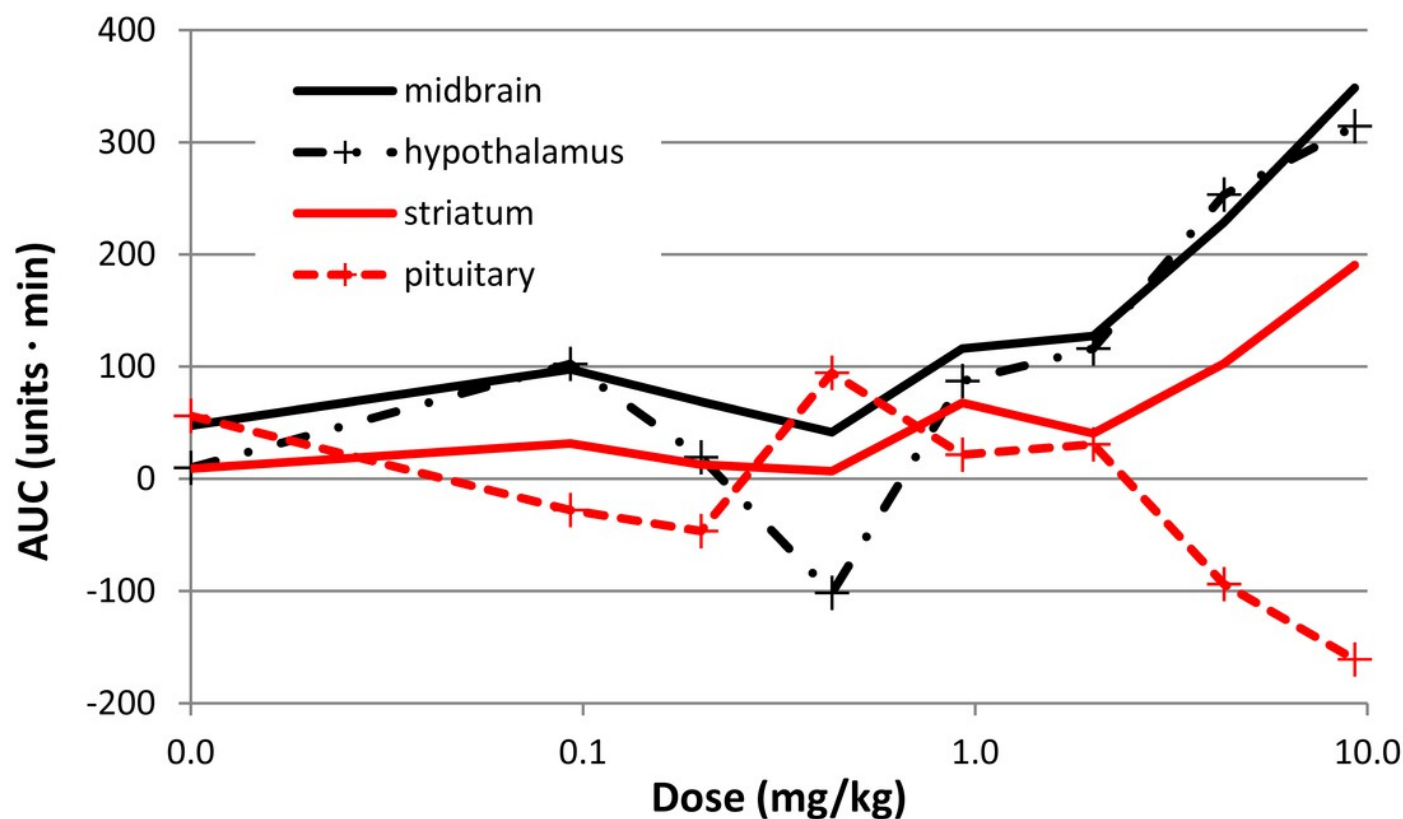
Figure 10

Time-BOLD curves for SKF38393, by dose

BOLD signal over time for the five highest doses of SKF38393. Each signal curve was detrended as described in the previous figure legend. The indicated dose of drug was given i.v. between the 15 and 20 minute marks.
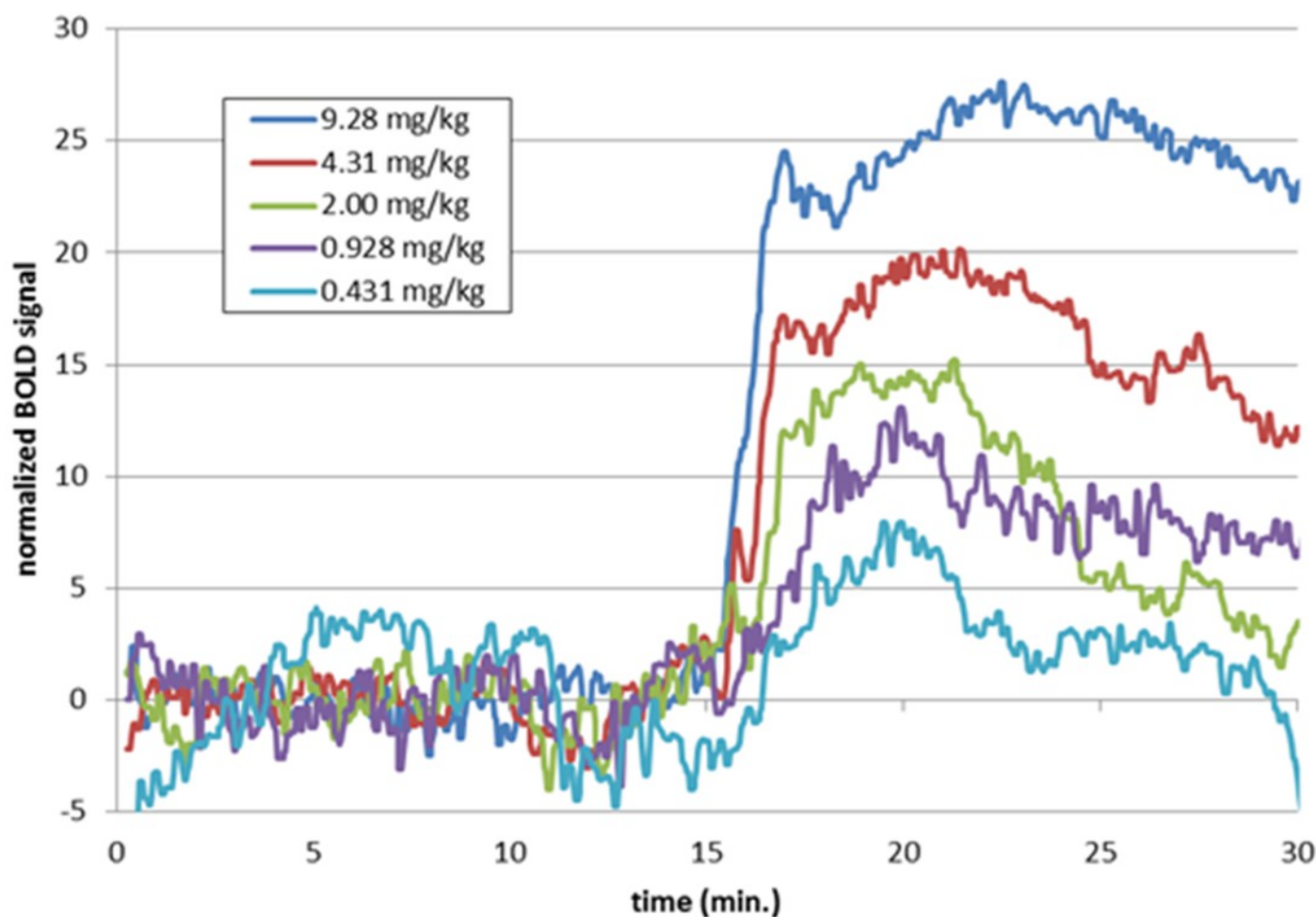
Table 1 (on next page)

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 ± S.D.	Increase in prolactin concentration with anesthesia
R	4.08	2.62 ± 0.35	354 %
T	4.25	3.04 ± 0.41	334 %
B	4.33	2.64 ± 0.34	236 %
E	6.75	4.05 ± 0.94	473 %
S	6.92	3.50 ± 0.96	1198 %
D	14.08	23.5 ± 6.02	410 %

Table 2 (on next page)

Responses to pramipexole by dose (N=1)

AUC_{15-30} 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 (AUC$_{15-30}$)	BOLD signal response, pituitary (AUC$_{15-30}$)	BOLD signal response, hypothalamus (AUC$_{15-30}$)	BOLD signal response, striatum (AUC$_{15-30}$)
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