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Decreasing prolactin levels leads to a lower diving effort but does not affect breeding success in Adélie penguins

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ABSTRACT

Current research on seabirds suggests a key role of hormones in the trade-off between self-maintenance and parental investment through their influence on foraging decisions during the breeding period. Although prolactin is known to have major effects on parental care, its role in foraging behavior has rarely been investigated in seabirds to date.

The aim of this study was to assess the influence of an experimental decrease in prolactin levels on foraging decisions and its consequences on breeding success in free-living seabirds. To achieve this, we implanted bromocriptine (an inhibitor of prolactin secretion) in male Adélie penguins (*Pygoscelis adeliae*), monitored their foraging behavior using time-depth recorders over several trips, and recorded their reproductive output.

On average 8 ± 0.5 days after implantation, we showed that bromocriptine administration led to an efficient decrease in prolactin levels. However, no differences were seen in foraging trip durations between bromocriptine-implanted birds and controls. Moreover, the time spent diving and the number of dives performed per trip were similar in both groups. By contrast, all diving parameters (including diving efficiency) were negatively affected by the treatment during the first at-sea trip following the treatment. Finally, the treatment did not affect adult body condition or chick growth and survival.

Our study highlights the short-term negative effect of low prolactin levels on diving effort, but indicates that a short-term and/or low-magnitude decrease in prolactin levels alone is not sufficient to modify consistently the body maintenance or the parental investment of Adélie penguins.

Introduction

Long-lived species should behave as 'prudent parents' during the breeding season, i.e. they should adjust their energy investment according to environmental constraints and parental capacity to minimize the risk of mortality while maximizing their future breeding opportunities (Drent and Daan, 1980). The context-dependent decision to initiate and/or continue a reproductive event, as well as the quantity of time and energy that will be invested has major consequences on the fitness of each individual. In this context, the role of endocrine processes in controlling life-history decisions deserves specific attention. Hormones are involved in the mediation of interactions between the environment and the behavioral and/or physiological responses of organisms (Ricklefs and Wikelski, 2002). This is particularly true for corticosterone (CORT) and prolactin (PRL), two hormones that are recognized as mediators of this conflict between self-maintenance and parental care in birds (reviewed in Angelier and Chastel, 2009), where they play opposing roles.

CORT is the main glucocorticoids in birds, and it promotes behaviors related to self-maintenance or survival in situations such as 'emergency states' (Wingfield et al., 1998). Basal levels of CORT are modulated according to the ratio of available energy to energy requirements (Landys et al., 2006). For example, an increase in basal CORT levels occurs in response to life-threatening perturbations, such as low available resources (e.g. Kitaysky et al., 1999), low levels of energy reserves (e.g. Spée et al., 2010) or periods of high energy requirements, e.g. reproduction (reviewed in Romero, 2002). It is also evident that CORT influences foraging behaviors (e.g. Angelier et al., 2007b, 2008, 2009a, 2009b; Crossin et al., 2012; Kitaysky et al., 2001). As an illustration of this, Angelier et al. (2007b) showed that post-trip CORT levels in breeding wandering albatrosses (*Diomedea exulans*) were negatively correlated to individual foraging success.

Conversely, PRL promotes the expression of parental care in both male and female birds (reviewed in Buntin, 1996). An increase in

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basal levels of PRL is observed following egg laying, with high concentrations being maintained throughout the incubation period in many species (Sockman et al., 2006). In altricial birds, the relatively high adult PRL levels following the hatching of their young persist until the chicks no longer need parental care (Garcia et al., 1996; Lormée et al., 2000). In seabirds, which perform foraging trips at sea throughout the breeding season, the maintenance of high PRL levels prompts individuals to return to their nests regularly for the feeding and protection of their offspring (Lormée et al., 1999; Vleck et al., 2000a). However, the relationship between PRL and parental behavior is complex and can be influenced by both intrinsic (such as the level of other hormones, or the breeding experience) and environmental factors (see Angelier and Chastel, 2009).

Recent studies provide strong evidence that CORT and PRL basal levels might be mechanistically linked (Angelier et al., 2009a). As such, high levels of CORT lead to a decrease in PRL, which has in turn been related to a low parental effort in several bird species (Angelier et al., 2007a, 2009a, 2009b; Criscuolo et al., 2005; Groscolas et al., 2008; Kitaysky et al., 2001; Spée et al., 2010; Wingfield and Kitaysky, 2002). For instance, exogenous CORT administration in long-lived birds decreased PRL levels and reduced breeding success (Angelier et al., 2009a, 2009b; Criscuolo et al., 2005). Although the effects of exogenous CORT on parental care have often been studied (e.g. Angelier et al., 2007a, 2009a, 2009b; Criscuolo et al., 2005; Crossin et al., 2012; Horton and Holberton, 2009; Kitaysky et al., 2001; Spée et al., 2011a), experimental studies examining the effects of a modulation of PRL levels on life-history decisions are rare (e.g. Angelier et al., 2006, 2009a; Reddy et al., 2002, 2007). Yet the effect of PRL on parental care is context-dependent and non-linear, leading some authors to suggest that further investigations are needed to better decipher the hormonal basis of parental investment in birds (Angelier and Chastel, 2009).

The present study experimentally decreased PRL levels in Adélie penguins (*Pygoscelis adeliae*) in order to examine the consequences on the trade-off between self-maintenance and reproduction. This was achieved with the use of bromocriptine, a PRL secretion inhibitor known to decrease PRL levels in many birds (Reddy et al., 2002, 2007) including King (*Aptenodytes patagonicus*, Jouventin and Mauget, 1996), emperor (*A. forsteri*, Angelier et al., 2006) and Adélie (Thierry et al., 2013) penguins. In addition, we used time-depth recorders (TDRs) to monitor the diving behavior of the study birds throughout several foraging trips. The consequences of treating adults with bromocriptine on chick growth and survival were also examined, both during and after the experimental study periods. As the literature on hormonal manipulations is broadly more detailed on male Adélie penguins than on females and because treating both partners could induce confounding effects or be deleterious for the current reproduction (see Cottin et al., 2011; Spée et al., 2010, 2011a, 2011b), we chose to manipulate only the PRL levels of males in this study. As central place foragers, the Adélie penguins are particularly energetically challenged during the breeding season. They indeed have to perform many trips between the at-sea feeding areas and the reproductive site in order to regularly supply food to their offspring. Maintaining high levels of PRL throughout the chick-rearing period (despite long periods spent at sea) would provide them with the motivation to return regularly on land to brood and feed their chicks (Lormée et al., 1999). Consequently, we predicted that decreasing basal PRL levels of male Adélie penguins would therefore have negative effects on their parental investment, and thus affect their motivation to brood and provision the chicks.

Methods

Study site and birds

Fieldwork took place during the 2010–2011 austral summer at Dumont d'Urville French Station, Adélie Land, Antarctica (66°40′S, 140°01′E). Sixty pairs of penguins were randomly marked with a Nyanzol-D number painted on the chest at the end of the courtship period, i.e. mid-November. Sexing was performed by a combination of parameters including cloacal inspection before egg-laying and observations of incubation behavior (see Beaulieu et al., 2010). Marked penguins were subsequently monitored several times a day to check the presence of each partner on the nest and to note the laying and hatching dates together with any breeding failures and egg/chick mortality.

At the beginning of the guard stage (i.e. mid-late December), 26 marked males with chicks were selected and divided into two experimental groups (Fig. 1A). The first experiment ($n = 10$) was designed to validate the effects of bromocriptine implants on PRL levels, whereas the second experiment ($n = 16$) enabled us to assess their effects on the diving behavior. Additionally, 8 marked birds were also monitored throughout the study period in order to control for instrumentation effects.

The protocol performed on Adélie penguins received the approval of the Ethics committee of the French Polar Institute Paul Emile Victor (IPEV) and authorizations were given by the TAAF (Terres Australes et Antarctiques Françaises); # 2010-79 of September, 3rd 2010 and 2010-67 of September, 3rd 2010. Moreover, all experiments were done in accordance with the rule of the European Committee Council Directive of November 24, 1986 (86/609/EEC) and the French Department of Agriculture (license no. 67-226 to T.R.).

Experimental protocol

Each bird in experiments #1 and #2 ($n = 26$) was captured twice during the study period. The first capture occurred in late December/early January (T0, Fig. 1A). Half of the birds ($n = 5$ for experiment 1 and $n = 8$ for experiment 2) were implanted with a bromocriptine pellet (Innovative Research of America, Sarasota, FL, USA). These implants are biodegradable pellets containing 25 mg of bromocriptine (hereafter B25). They are designed for a 21-day-release in rodents and we have previously tested these implants, along with other implant doses (0.5, 5, 10 mg of bromocriptine), in Adélie penguins (Spée et al., unpublished work). We implanted the pellet under the skin in the nape of the neck through a small incision (ca. 0.5–1 cm), which was then sutured with two sterile stitches and sprayed with Alumisol ® (aluminum powder, healing external suspension). Sham-implanted individuals (hereafter 'Controls') underwent the same procedure, undergoing incision but without the pellet implantation. All the birds in experiment 2 were also equipped with TDRs (see details in the 'diving behavior recording' section). All the study birds and their chicks were weighed using an electronic balance (Ohaus, ±2 g) and adult flipper lengths were measured using a ruler (±1 mm). An index of body condition (BCI) was calculated as follows: $BCI = bm/l^3 \times 10^7$, where 'bm' is the body mass in kg, and 'l' is the flipper length in mm (Cockrem et al., 2006). Birds were released near their nest and visual observations were made every two hours throughout the implantation period (approximately 2 weeks) to determine which birds were present on the nest and to measure foraging trip duration.

At the end of the experiment (on average 8 ± 0.5 and 14 ± 0.4 days after implantation for experiments 1 and 2, termed T1 and T2 in Fig. 1A, respectively), all birds were recaptured on their nest after several foraging trips at sea. The TDRs were recovered and body mass and flipper lengths were measured in adults and chicks. At this time, two small plastic markers (Fishtags, FloyTag, Seattle, WA, USA) were attached to the backs of chicks in experiment 2 in order to facilitate future identification, notably during the crèche stage (T3).

Immediately after each capture (T0, T1 & T2), approximately 1–2 ml of blood was collected from the flipper or the tarsus veins. As recommended by Vleck et al. (2000b), sample collection was carried out in less than 5 min. Samples were transferred into heparinized vials and then centrifuged (10 min at 4 °C, 5000 rpm). Plasma and red blood cells were stored separately at −20 °C until assayed. For experiment 2, the overall handling lasted between 15 and 24 min (on average

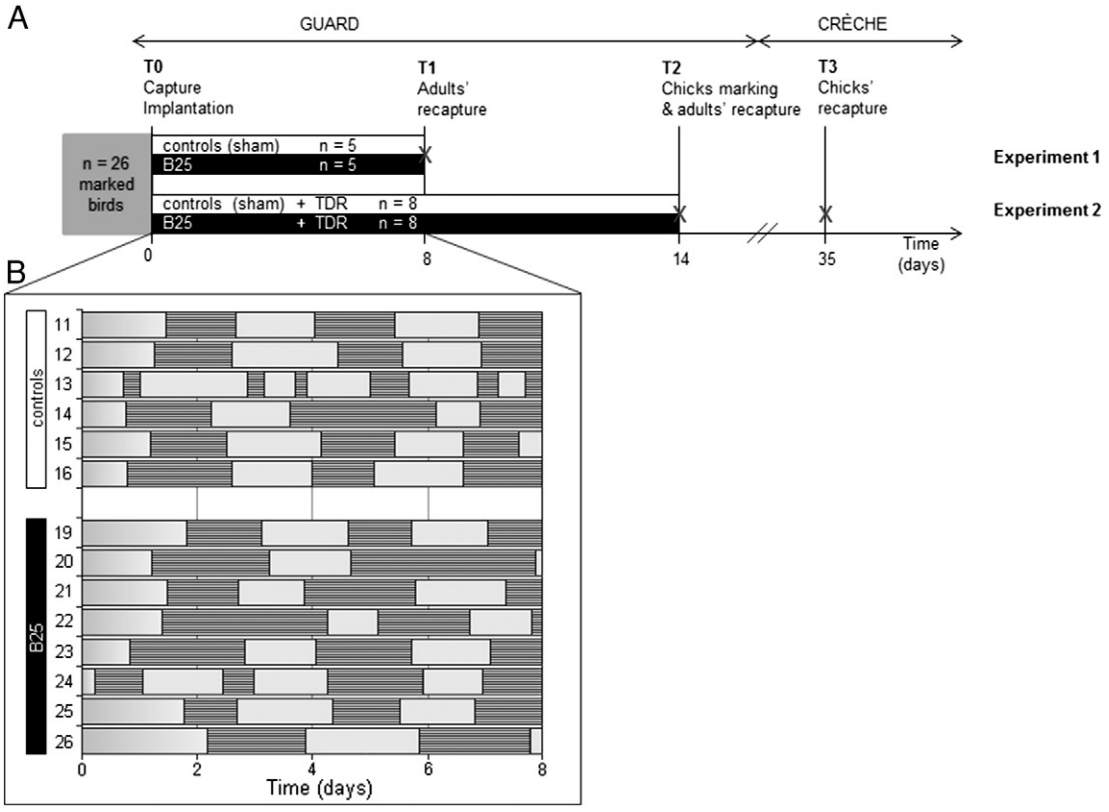

Fig. 1. A. Study protocol applied to 26 male Adélie penguins during the guard and crèche stages. Experiment 1 ran from T0 to T1 and Experiment 2 from T0 to T2. See the Methods section for more details. B. Alternation between nesting bouts (gray bars) and at-sea foraging trips (hatched bars) of controls (n = 6) and B25-treated (n = 8) birds of Experiment 2 during the 8 days following the treatment.

19 min) during the first capture (i.e. capture, blood sampling, implantation, equipment and measures) and between 10 and 26 min (on average 17 min) on recapture (capture, blood sampling, removing TDRs and measures).

In order to evaluate the effects of treatment on offspring, 41-day-old chicks from experiment 2 were recaptured during the crèche stage, i.e. on average 35 days after the capture of their parents (T3, Fig. 1A). A blood sample (only 500 μl) was collected from the tarsus vein and stored as whole blood at −20 °C. Their body mass and flipper lengths were measured and their plastic markers were removed.

Diving behavior

To determine the diving activity of the 16 adult males (experiment #2), miniature TDRs (M190-DT: 49 × 15 mm, 14 g; and M190L-DT: 52 × 15 mm 16 g; Little Leonardo, Co., Tokyo, Japan) were attached to the penguins' backs using mastic and strips of waterproof black Tesa® tape (Beiersdorf AG, Hamburg, Germany) (Wilson et al., 1997). These TDRs recorded temperature and depth every 1 s (5 cm resolution) on a 32MBit memory. Data were analyzed using the IGOR Pro software (Wavemetrics, version 6.12A, Portland, USA) and surfacing was carried out using the 'WaterSurface_D2GT' program on the 'Ethographer' application (Sakamoto et al., 2009). Diving parameters such as maximum dive depth, dive and bottom durations, number of undulations and post-dive interval duration (see Ropert-Coudert et al., 2007 for definitions) were extracted automatically for each dive using a custom-made program in IGOR Pro. The post-dive interval duration was calculated as the time spent at the surface between two successive dives. Only dives >1 m were included in the analysis according to the resolution of the TDR and to avoid the noise at sea-surface. During the bottom phase, penguins performed undulation events, which can be used as an index of the number of pursued preys (Bost et al., 2007), such as krill or fish. Diving efficiency was calculated as bottom time / (dive duration + post-dive interval duration) (Kooyman et al., 1992). Trip duration was calculated as the total time elapsed between the first and the last dive recorded by TDRs (see Fig. 1B for the alternation between foraging trips and nesting bouts among individuals of both groups).

Assessment of instrumentation effect

In order to assess instrumentation effects, we monitored the duration of foraging trips in 8 unequipped controls and then compared them with those of sham-implanted birds equipped with TDRs from experiment 2 (n = 8). The five trips following the equipment of animals were taken into account, and trip duration was assessed by two-hourly visual observations of the nest for both experiments. We found no effect of instrumentation on foraging trip duration (GLMM instrumentation × trip rank: $F = 0.3$, $df = 53$, $p = 0.9$).

Hormone assays

Plasma concentrations of PRL were determined by a heterologous radioimmunoassay (RIA) at the Centre d'Etudes Biologiques de Chizé (CEBC, Villiers en Bois, France) as detailed in Cherel et al. (1994). Pooled plasma samples of Adélie penguins produced a dose–response curve that paralleled chicken PRL standard curves ('bAFP 4444BQ', source: Dr. Parlow, National Hormone and Peptide Program Harbor — UCLA Medical Center, Torrance, California, USA). The minimum detectable PRL levels are ~5 $ng \cdot ml^{-1}$, and no samples were below this limit.

Plasma concentrations of CORT were determined in our laboratory (DEPE-IPHC, Strasbourg, France) by enzyme-immunoassay (AssayPro, AssayMax Corticosterone ELISA Kit, St. Charles, Missouri, USA). The minimum detectable dose of this hormone with this kit is typically

~0.3 ng·ml^{-1} and the cross-reactivity of the CORT antibody with other steroids is low (range between 0 and 2%, Assay Pro). CORT levels were considered as baseline values since no relationship was found between handling time and CORT concentrations (Capture, all groups: $n = 25$, $F < 0.01$, $t = 0.03$, $p = 0.98$, $r^2 < 0.01$; Recapture — Controls: $n = 12$, $F = 0.96$, $t = 0.98$, $p = 0.35$, $r^2 = 0.09$).

All samples were run in duplicate. Intra- and inter-assay variations were 6% and 9% for CORT and 5% and 13% for PRL, respectively.

Stable isotope analysis

Stable isotope signatures of nitrogen (N) and carbon (C) are widely used to estimate the trophic level of the prey ingested and the foraging habitat (coastal vs. offshore) of organisms (Cherel and Hobson, 2007). This tool has recently been used to investigate whether Adélie penguins switched to more available prey like coastal fish according to sea-ice conditions (Beaulieu et al., 2010). Adélie penguins are known to mainly feed on a combination of fish (*Pleuragramma antarcticum*) and krill (*Euphausia superba* and *E. crystallorophias*) in Adélie Land (Wienecke et al., 2000). Tierney et al. (2008) found that the isotopic signatures of Adélie penguins were relatively consistent with the identification of prey found in their stomachs.

Because the isotopic signature depends on the selected tissue and the duration of its complete turnover (Bearhop et al., 2002), we examined the plasma (turnover of approximately 3 days) of birds from experiment #1 (recaptured ≈ 8 days after treatment) and the red blood cells (turnover of approximately 1 month) of experiment #2's individuals (recaptured ≈ 14 days after treatment) (Hobson and Clark, 1993). Analyses for chicks were performed on whole blood due to the small quantity of blood collected.

Only plasma samples were delipidated with cyclohexane (Cherel et al., 2010), since the low lipid content of blood does not require extraction for stable isotope analyses (Cherel et al., 2005). All samples were then lyophilized for 48 h, powdered, weighed with a microbalance (range: 0.4 to 1.3 mg) and transferred into capsules for analyses at the LIttoral ENvironnement et Sociétés (LIENSs, La Rochelle, France). Replicate measurements showed coefficients of variation for $\delta^{13}C$ and $\delta^{15}N$ values of standard acetanilide of ± 0.1% and ± 4.6%, respectively. Values are expressed in the usual δ notation (‰) relative to PeeDee Belemnite (PDB) for $\delta^{13}C$ and atmospheric nitrogen (N_2) for $\delta^{15}N$. One outlier was removed from analyses (1 control adult from experiment #2).

Statistics

Statistical analyses were performed using R 2.14.2 (R Development Core Team 2008). Values are presented as means ± SE and alpha levels were set at $\alpha = 0.05$. Student t-tests were used for comparisons of means between control and B25-treated birds and paired t-tests were used to compare different stages (e.g. T0 vs. T1) within one group.

For the analyses of diving parameters (dive and bottom durations, number of undulations, dive efficiency, and post-dive interval durations), we used General Linear Mixes Models (GLMM, 'nlme' package in R) with individuals as random factors, allowing us to take repeated measures into account, as birds were monitored over several successive dives. Moreover, the maximum depth reached by penguins during each dive was added as a covariate in the model since it affects all dive parameters. p-Values were then obtained by applying an analysis of variance (ANOVA) on the model. Cohen's effect size estimates (*d*, Cohen, 1988) were calculated online (www.cognitiveflexibility.org) using the average mean and standard deviation (SD).

For all statistical tests, the normality of the data was tested (with a Shapiro–Wilk test for t-tests and with a QQplot of the residuals of the model for GLMM). If data did not follow the normal distribution, a log-transformation was applied.

For stable isotope analyses, Multivariate Analyses of Variance (MANOVAs, with λ Wilk's tests) were used to compare the overall diet signature (binding $\delta^{13}C$ and $\delta^{15}N$) between both groups.

Results

Experiment 1

Birds from experiment 1 were recaptured on average 8 ± 0.5 days after implantation to validate the effects of bromocriptine implants on plasma PRL levels. At T0, PRL concentrations averaged 52 ± 4 ng·ml^{-1} and were not significantly different between the two groups ($t = 0.03$, $df = 5$, $p = 0.98$) (Fig. 2A). In addition, PRL levels in controls on capture (T0) were not significantly different to those recorded on recapture (T1) (paired t-tests: $t = 1.2$, $df = 4$, $p = 0.3$). Conversely, B25-treated birds showed significantly lower PRL levels than controls on recapture (T1) ($t = 2.8$, $df = 6$, $p = 0.03$), reaching on average 29 ± 5 ng·ml^{-1} (Fig. 2A).

At T0, basal CORT levels were on average 4.3 ± 0.4 ng·ml^{-1} and were similar between both groups ($t = 1.9$, $df = 3$, $p = 0.2$; Fig. 2B). At T1, CORT levels were slightly higher for B25-treated birds than controls ($t = 3.2$, $df = 7$, $p = 0.02$), however the difference between both groups was only 2.8 ng·ml^{-1} on average.

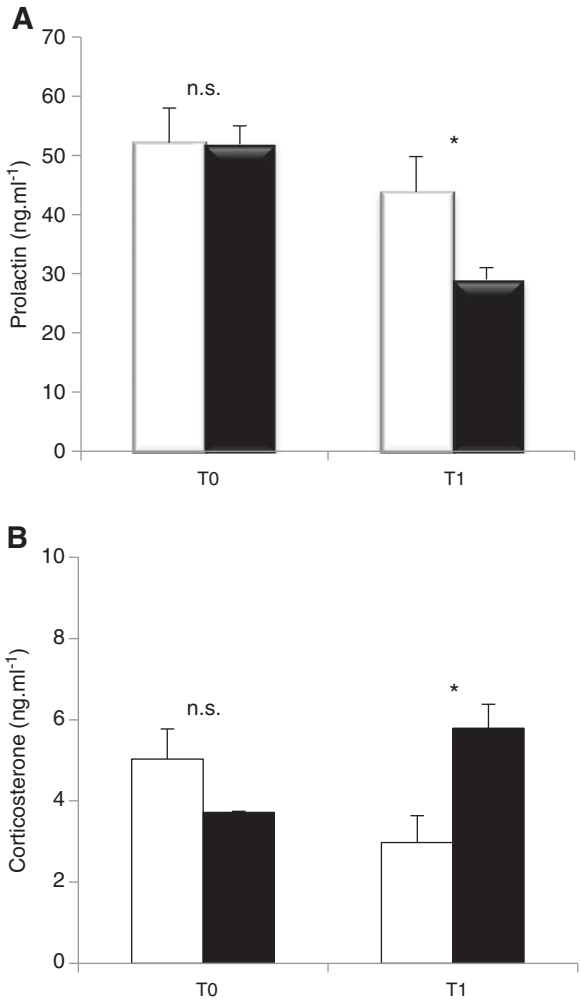

Fig. 2. Effects of bromocriptine implants (B25) on the plasma levels (mean ± SE) of prolactin (A) and corticosterone (B) for controls (white bars, $n = 5$) and B25-treated (black bars, $n = 5$) male Adélie penguins. T0 and T1 are the beginning and the end (on average 8 ± 0.5 days after implantation) of the experiment 1, respectively. 1 outlier was removed for corticosterone measurement of one control individual. * indicates a significant difference ($p < 0.05$) between B25 and control groups. n.s.: non-significant.

Experiment 2

At T0, no significant difference was observed between controls and B25-treated birds for adult body condition, chick body mass or adult hormone levels (Table 1). All 16 birds continued to breed normally after the experimental procedure and all the TDRs were recovered at the end of the experiment (T2). In the control group, two TDRs failed to record data.

Effects of B25-treatment on foraging behavior

During the period in which individuals were equipped with TDR (on average 14 ± 0.4 days), males conducted on average 4.4 ± 0.4 foraging trips (ranging from 3 to 9 trips). This was similar in both control and B25-implanted birds ($t = 1.3$, $df = 7$, $p = 0.2$).

Because we found in experiment 1 that the decrease of PRL was effective at least during the 8 days following implantation, the analyses of foraging behavior were performed only during this period. Durations of foraging trips and nesting bouts following implantation/equipment are shown on Fig. 1B. Time before departure for the first foraging trip did not differ significantly between control and B25-treated males ($mean_{control}$: 1.0 ± 0.1 day; $mean_{B25}$: 1.4 ± 0.2 days; $t = 1.3$, $df = 11$, $p = 0.2$; first gray rectangles in Fig. 1B). Moreover, the percentage of time spent at sea was similar between both groups ($mean_{control}$: 46 ± 5%; $mean_{B25}$: 51 ± 3% of time spent at sea; $t = 0.7$, $df = 9$, $p = 0.5$).

Because individuals did not perform the same number of trips during this period (from 2 to 5.5, Fig. 1B), our diving analyses focused on the first at-sea trip following implantation.

At the level of the trip, no parameters (trip duration, number of dives performed and time spent underwater) were significantly different between control and B-25 treated birds (Table 2). However, at the level of the dive, B25-treated males reached on average significantly lower maximum dive depths than controls. Moreover, all dive parameters analyzed in this study (dive and bottom durations, number of undulations, and dive efficiency) were negatively affected by the treatment (Table 2). The post-dive interval duration was not affected by the treatment ($mean_{control} = 58 \pm 7$ s, $mean_{B25} = 107 \pm 38$ s; GLMM: $F = 1$, $df = 15{,}864$, $p = 0.5$).

Effects of B25-treatment on adults' body condition, hormonal levels and on their chicks

CORT levels of both controls and B25-treated birds did not change significantly between capture (T0) and recapture (T2) (paired t-tests; control: $t = 0.4$, $df = 7$, $p = 0.7$; B25: $t = 0.3$, $df = 7$, $p = 0.8$). However, PRL levels were significantly lower in both groups at the end of the experiment (T2) (paired t-tests; control: $t = 2.6$, $df = 6$, $p = 0.04$; B25: $t = 4.8$, $df = 7$, $p = 0.002$). PRL levels decreased by 21 and 28% on average for controls and B25-treated birds, respectively. At the end of the experiment (T2), control and B25-treated birds exhibited similar body conditions and hormone levels (PRL and CORT, Table 1).

At the beginning of the experiment (T0), there were 12 and 13 chicks in control and B25-treated birds groups, respectively. At T2, two chicks from control groups had died and all B25-treated birds had only one chick per pair (i.e. 5 chicks from B25 group died). Only one chick per group died between T2 and T3. Moreover, the mean brood mass per pair was never significantly different between groups (Table 1).

B25-treatment did not have a significant effect on the chick growth (Fig. 3), as relationships between body mass and flipper length did not differ significantly between groups (ANOVAs: T2: $F < 0.01$, $df = 1$, $p = 0.9$; T3: $F = 1.2$, $df = 1$, $p = 0.3$).

Effects of B25-treatment on diet composition

No significant difference was obtained between the overall isotopic signature of control and B25-treated adults at T1 (MANOVA, Wilk's λ, $F = 0.2$, $df = 6$, $p = 0.8$) or at T2 (MANOVA, Wilk's λ, $F = 0.1$, $df = 13$, $p = 0.9$) (Fig. 4). Differences observed between T1 and T2 may be due to the different tissues (plasma or red blood cells) used for stable isotopic analyses. Moreover, neither $\delta^{13}C$ nor $\delta^{15}N$ values of chicks from experiment 2 were significantly affected by the treatment applied to the male (MANOVA, Wilk's λ, $F = 0.01$, $df = 13$, $p \approx 1$).

Discussion

Bromocriptine (B25) implants led to an efficient decrease in PRL basal levels in male Adélie penguins during the chick-rearing period (T1, on average 8 days following implantation), followed by a recovery at the end of the experiment (T2, on average 14 days following implantation). Although this transient PRL decrease was associated with some subtle changes in foraging behavior, especially regarding the diving effort, no noticeable effect was found on breeding success, at least during the 41 days after hatching.

If low PRL levels decrease parental investment, then we could have expected low PRL birds to conduct longer foraging trips than controls. This hypothesis has already been tested in black-legged kittiwakes (*Rissa tridactyla*), where birds with low PRL levels spent more time at sea, resulting in lower nest attendance, and breeding success (Angelier et al., 2009a). Indeed, Chaurand and Weimerskirch (1994) proposed that seabirds promote self-maintenance when conducting long trips, as they restore their body reserves while reducing chick provisioning. Yet, a PRL decrease did not have any impact on the foraging trip duration and time spent diving during trips in our treated Adélie penguins. Additionally, the treatment had no effect on the stable isotopic signature of both adults and their chicks, suggesting that birds from both groups forage at similar trophic levels and in the same areas. Similarly, Crossin et al. (2012) recently showed that the regulation of foraging

Table 1
Comparisons between controls and B25-treated male Adélie penguins at the beginning (T0) and at the end (T2) of experiment 2. Values of the brood mass after the end of the experiment (T3) were also added.

Parameters	Stage	Control (n = 8)	B25 (n = 8)	t	df	p
Flipper length (cm)	T0	19.2 ± 0.2	19.6 ± 0.2	1.1	14	0.3
Body condition index	T0	6.4 ± 0.3	6.7 ± 0.1	0.8	10	0.4
	T2	6.0 ± 0.2	6.4 ± 0.2	1.4	14	0.2
Brood mass (g)	T0	392 ± 55	514 ± 63	1.5	14	0.2
	T2	1573 ± 171	1354 ± 86	1.1	10	0.3
	T3	4046 ± 458	3449 ± 258	1.1	13	0.3
Brood age (days)	T0	4.9 ± 0.6	5.8 ± 0.8	1.0	13	0.3
Prolactin levels ($ng \cdot ml^{-1}$)	T0	56.7 ± 2.9[a]	54.2 ± 3.1	0.6	13	0.6
	T2	44.5 ± 4.4[a]	38.5 ± 2.0	1.5	10	0.2
Corticosterone levels ($ng \cdot ml^{-1}$)	T0	4.0 ± 0.4	4.0 ± 0.9	~0	10	~1
	T2	4.8 ± 1.1	4.3 ± 1.7	0.9	13	0.4

Results are expressed as means ± SE. The brood mass is equal to the sum of the body masses of the chicks (1 or 2).
[a] 1 outlier removed.

Table 2
Foraging patterns of controls and B25-treated male Adélie penguins during the first at-sea trip following the implantation.

Parameters	Treatment		Cohen's *d* (effect size)	Statistics		
	Control (n = 6)	B25 (n = 8)		t	df	p
Trip scale						
Trip duration (days)	1.25 ± 0.21	1.61 ± 0.24	−0.605	1.1	12	0.3
Number of dives	950 ± 169	1275 ± 257	−0.570	1.1	11	0.3
Time spent underwater (h)	14.4 ± 2.6	14.4 ± 2.6	0.000	~0.0	12	~1.0
				F	df	p
Dive scale						
Maximum depth (m)	17.3 ± 1.8	10.6 ± 1.5	1.564	8	12	**0.014**
Dive duration (s)	56 ± 4	41 ± 5	1.250	43	15,878	<**0.001**
Bottom duration (s)	34 ± 3	25 ± 3	1.091	16	15,878	<**0.001**
Number of undulations	4.8 ± 0.5	3.5 ± 0.3	1.250	10	15,878	**0.002**
Efficiency	0.42 ± 0.02	0.41 ± 0.03	0.163	50	15,864	<**0.001**

Results are expressed as means ± SE. Parameters measured at the trip scale were tested with t-tests, whereas parameters at the diving scale were tested with General Linear Mixed Models (GLMM), allowing taking into account repeated measures (successive dives) as well as the maximum dive depth as a covariate. Bold values indicate significant differences ($p < 0.05$) between both groups. Efficiency was calculated as bottom time / (dive duration + post-dive interval duration), see Methods section for more details.

behavior of macaroni penguins (*Eudyptes chrysolophus*) was not affected by the variation of PRL levels. On the other side, these authors highlighted the positive influence of high levels of CORT on foraging and diving activities, as well as on chick mass. In our study, proximate levels, i.e. dive parameters, seem to be more affected by the manipulation of PRL levels. Thus, behavioral changes were observed at the dive scale, with bromocriptine treatment leading to a lower foraging effort with lower at-sea efficiency. Accordingly, low PRL levels might have triggered a decrease in the birds' motivation to catch food for themselves and/or for their chicks. This finding is in accordance with previous literature indicating that high PRL levels would promote the provisioning of offspring in birds (Duckworth et al., 2003; Lormée et al., 1999; Vleck et al., 2000a; Wang and Buntin, 1999). However, contrary to our prediction, the decreasing PRL levels in our study had no significant effects on either adult body condition or breeding success since the growth and survival of chicks were similar between controls and B25-treated birds, at least over a short-term scale. Several non-exclusive hypotheses can be proposed to explain the absence of effects on parental investment observed in our study.

(1) The decrease in PRL levels might be too brief to induce negative effects on chicks. Thus, our results indicate that PRL basal levels had lowered after 8 days but reached similar values than those seen in controls after 14 days. Although these implants were originally designed to diffuse bromocriptine in rodents over a 21-day period, it has already been demonstrated that they can deliver the drug over a shorter period than expected when used in birds (see the example of CORT implants diffusion on Eurasian kestrels, *Falco tinnunculus*, and barn owls, *Tyto alba*, in Muller et al., 2009). In our study, the decrease in PRL levels was only efficient less than two weeks following the bromocriptine pellet implantation. In a recent study performed in free-living incubating Adélie penguins and using the same pellets, it has been reported that PRL levels were still significantly decreased in bromocriptine-treated birds 19.5 days after implantation compared with control birds, thus indicating a role of the life-history stage in the response to treatment (Thierry et al., 2013).

(2) The magnitude of the decrease in PRL levels may not be strong enough. This hypothesis would agree with the findings of Angelier and Chastel (2009) who proposed that PRL levels must reach a minimum threshold value before inducing negative effects on parental behavior. Yet, Spée et al. (2010) suggested that a low threshold of 30 $ng \cdot ml^{-1}$ PRL levels leads to nest abandonment behavior in male Adélie penguins during the incubation fast. In our study, about 8 days after implantation, B25-treated birds showed 34% lower PRL values than controls birds, reaching 29 ± 5 $ng \cdot ml^{-1}$ (against 44 ± 2 $ng \cdot ml^{-1}$ for controls, Fig. 2A). At such a low level, our birds should have abandoned their nests, suggesting that the relationship between PRL

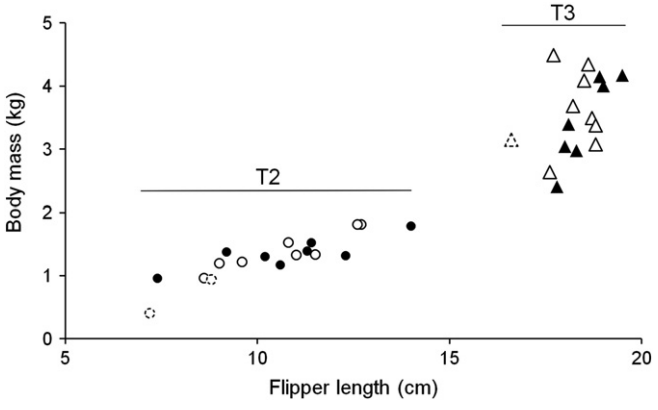

Fig. 3. Relationship between body mass (kg) and flipper length (cm) of chick from controls (open symbols) and B25-treated (closed symbols) male Adélie penguins. Chicks at T2, i.e. end of the experiment 2 (on average 14 ± 0.4 days following implantation) are indicated by circles, while chicks at T3, i.e. recapture of 41 days old chicks (crèche stage) are represented by triangles. Symbols in dotted lines correspond to values for the second chick of a two-chick pair. Note that only one of the two second chick survived from T2 to T3.

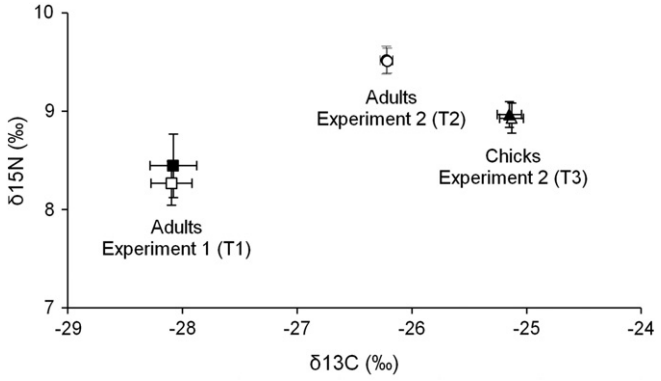

Fig. 4. $\delta^{15}N$ and $\delta^{13}C$ values (means ± SE) of controls (open symbols) and B25-treated (closed symbols) male Adélie penguins at T1 for adults from experiment 1, T2 for adults from experiment 2 and T3 for their chicks. Analyses were performed on plasma, red blood cells and total blood, respectively, according to tissue turnover and experiment constraints (see Methods section for details).

and parental behavior is much more complex than expected. Penguins in the study of Spée et al. (2010) were in a totally different breeding stage and nutritional state from ours, as they had already fasted for several weeks on the nest, reaching very low body conditions and high CORT levels (i.e. until the phase III of starvation).

(3) A decrease in PRL alone might also not be sufficient to induce negative effects on parental investment. The simultaneous presence of high CORT and low PRL levels in the blood could be seen as an 'adaptative' phenomenon, since reaching low PRL concentrations could terminate their current breeding attempt, and should therefore only be activated in an emergency situation. In our study, baseline CORT levels were similar between both groups, and all birds were far from a low threshold in their body reserves, so the B25-treated birds would not have been in this life-threatening situation. This hypothesis supports the current assumption that these two hormones with opposite effects could be strongly linked and could act together in trade-off mediation between reproduction and self-maintenance (Angelier and Chastel, 2009). This would also support the findings of a previous study dealing with the hormonal control of nest abandonment reporting that low PRL was associated with high CORT levels in deserting birds (Spée et al., 2010).

(4) It is also possible that some compensation phenomena occurred in our study, thus masking the effects of PRL on parental effort. Although some behavioral changes were observed during the first trip following the treatment, B25-treated males may have increased their foraging effort in subsequent at-sea trips. On an individual level, the presence of chicks on the nest might play a role as a 'proximate driver' for parental investment, acting as a strong external stimulus. As far as pairs are concerned, it is also possible that females compensate for the negative behavior of their mates, as already observed in many bird species (e.g. Wright and Cuthill, 1990). Yet a recent study on Adélie penguins did not show any compensatory behavior by partners (Beaulieu et al., 2009). Indeed, these authors showed that when the breeding workload of the member of a pair was increased through a handicapping procedure, the handicapped birds foraged longer and raised smaller chicks than controls, and no visible modification of their partners' behavior was noted. However, the fact that females did not compensate does not definitively exclude possible differences in their at-sea efficiency. Further studies should therefore simultaneously monitor males and females within the same pair using data loggers to examine whether females increase their foraging effort at sea when their males are hormonally treated.

(5) Finally, changes in PRL levels can also affect metabolic homeostasis by regulating key proteins associated with glucose and lipid metabolism in various target tissues on peripheral and central sites (Ben-Jonathan et al., 2006). Chronic elevation of PRL in rodents has been associated with increased food intake and body weight (Byatt et al., 1993; Moore et al., 1986). Conversely, the suppression of PRL release by bromocriptine resulted in the opposite trend (Bonomo et al., 2005; Knudtzon et al., 1986). Moreover, intracranial injections of PRL have also been shown to elevate food intake in ring doves (*Streptopelia risoria*) by promoting a selective and long-lasting hyperphagia (Buntin, 1989). While there is no clear evidencefor any effect on prey consumption in our study following bromocriptine treatment, it would be interesting to explore how the food intake of penguins can be affected by PRL levels, independently of any changes in CORT levels.

Our study highlights the negative effects of low PRL levels on foraging effort but indicates that a short-term and/or low-magnitude decrease in PRL levels alone is not sufficient to consistently modify the breeding success of male Adélie penguins. Further studies simultaneously examining the relationships between PRL and foraging effort, and its link with CORT levels, are crucial for a better understanding of the role played by these two hormones in controlling foraging decisions and modulating the trade-off between self-maintenance and reproduction.

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