Animal Behaviour 101 (2015) 179–189

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Evidence for a mechanism of phenotypic integration of behaviour and innate immunity in a wild rodent: implications for animal personality and ecological immunology

Andy Dosmann*, Katherine C. Brooks [1], Jill M. Mateo [2]

Committee on Evolutionary Biology, The University of Chicago, Chicago, IL, U.S.A.

ARTICLE INFO

Article history:
Received 5 August 2014
Initial acceptance 8 September 2014
Final acceptance 25 November 2014
Available online 28 January 2015
MS. number: A14-00637R2

Keywords:
animal personality
bacteria-killing ability
behavioural syndrome
ecological immunology
glucocorticoid receptor
innate immunity
phenotypic plasticity
stress response

If a single mechanism influences multiple traits, it may facilitate functional integration or impede optimal trait expression to produce consistent individual differences and correlations among those traits. The fields of animal personality and ecological immunology each aim to understand variation and covariation of behavioural and immune traits. Studying these traits together may provide additional insight into patterns of (co)variation than studying behaviours or immunity in isolation, as trade-offs between behaviour and immunity are likely. Hormonal mechanisms may be involved in the variation and covariation between behavioural and immune traits, and the role of receptors in particular has rarely been tested in wild animals. In wild-caught Belding's ground squirrels, *Urocitellus beldingi*, we delivered mifepristone to experimentally block the actions of glucocorticoid receptors (GRs), a component of the stress response. Then we evaluated whether cortisol binding with GRs affects the plasticity of behavioural and immune traits, consistent individual differences and phenotypic integration of exploratory behaviour, activity, antipredator behaviour, response to restraint and bacteria-killing ability, a measure of innate immunity. Mifepristone treatment abolished relationships between faecal glucocorticoid metabolite levels and both exploratory behaviour and bacteria-killing ability. This result indicates that cortisol binding with GRs is a mechanism of plasticity of those traits. Mifepristone also affected relationships among traits. Specifically, mifepristone treatment significantly modulated the relationships between bacteria-killing ability and two behaviours, exploration and activity. This result supports the hypothesis that the GR–cortisol binding is a mechanism of phenotypic integration. Together, these results suggest that GR–cortisol binding balances the often observed trade-off between behaviour and immunity to produce patterns of (co)variation of behavioural and immune traits seen in nature.

An individual's behavioural traits can vary from moment to moment in response to environmental change (i.e. phenotypic plasticity; Pigliucci, 2001; West-Eberhard, 1989). This has led to extensive investigation of the reasons why these traits are often correlated and show consistent individual differences over time and across environments (Dingemanse, Kazem, Réale, & Wright, 2010; Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Sih, Bell, & Johnson, 2004). Such correlations (i.e. behavioural syndromes) and consistent individual differences in behaviour (i.e. animal personality) correspond to similar patterns of variation of immune traits described by the field of ecological immunology (Ardia, Parmentier, & Vogel, 2011; Schmid-Hempel, 2003; Sheldon & Verhulst, 1996). Researchers have questioned whether physiological mechanisms are responsible for these patterns of (co)variation, but the extent to which such mechanisms influence behavioural and immune variation and covariation remains unresolved (Ardia et al., 2011; Demas, Adamo, & French, 2011; Duckworth & Sockman, 2012; Garamszegi et al., 2012; Koolhaas, 2008; Krams et al., 2013; Sih et al., 2004). If multiple traits share a single mechanism, then that shared mechanism can facilitate functional integration of those traits or impede their independent expression, analogous to the pleiotropic effects that a single gene may have on multiple traits (Duckworth & Sockman, 2012; Garamszegi et al., 2012; Ketterson & Nolan, 1999; Krams et al., 2013). By this reasoning, a number of traits may be relevant, but in this study we focus on relationships between behaviour and

* Correspondence and present address: A. Dosmann, Stanford University, 590 Escondido Mall, Stanford, CA 94305, U.S.A.
E-mail address: dosmann1@gmail.com (A. Dosmann).
[1] K. C. Brooks is now at the Department of Ecology, Evolution and Environmental Biology, 10th Floor Schermerhorn Extension, 1200 Amsterdam Avenue, New York, NY 10027, U.S.A. *E-mail address*: kcraig@gmail.com (K. C. Brooks).
[2] *E-mail address*: jmateo@uchicago.edu (J. M. Mateo).

http://dx.doi.org/10.1016/j.anbehav.2014.12.026
0003-3472/

immunity because immunity is relatively understudied with respect to animal personality (but see Kluen, Siitari, & Brommer, 2014; Krams et al., 2013; Sild, Sepp, & Hõrak, 2011) and both are central to other hypotheses of trait covariation and maintenance of variance (e.g. Ezenwa, Stefan Ekernas, & Creel, 2012; L. B. Martin, Brace, Urban, Coon, & Liebl, 2012; Rubenstein & Hauber, 2008). Here we investigated whether a single physiological mechanism influences behaviour and immunity of Belding's ground squirrels, *Urocitellus beldingi*, to produce phenotypic integration (i.e. a behavioural syndrome involving immunity). This will help clarify whether a mechanism accounts for the variability in behaviour and immunity that the fields of animal personality and ecological immunology aim to explain.

The stress response is part of the physiological reaction of individuals to environmental and social challenges, making it a likely mechanism of change in many traits, including behavioural and immune traits. When addressing the physiological stress response, researchers often manipulate and measure glucocorticoids, usually corticosterone or cortisol depending on the species, which are steroid hormones produced by the adrenal glands (Cockrem, 2007; Reeder & Kramer, 2005). Glucocorticoids mobilize energy, regulate immune and reproductive systems, and influence behaviour (Cockrem, 2007; Demas et al., 2011; Reeder & Kramer, 2005; Sapolsky, Romero, & Munck, 2000). By this account, it seems inevitable that environmental stimulation of glucocorticoid release will have wide-ranging effects. However, many of these studies have focused on one type of trait, while research on glucocorticoid-associated phenotypic integration of suites of traits reveals that phenotypic independence is not uncommon (e.g. Buehler et al., 2012; Garamszegi et al., 2012; Koolhaas, De Boer, Coppens, & Buwalda, 2010). This may be because the actions of glucocorticoids have multiple pathways (Sapolsky et al., 2000). After release in response to a real or perceived environmental challenge, glucocorticoids can exert nongenomic effects, but most often bind with two types of receptors that initiate transcription (Groeneweg, Karst, de Kloet, & Joëls, 2011; Sapolsky et al., 2000). Mineralocorticoid receptors (MRs) bind to glucocorticoids with high affinity and are nearly saturated at baseline levels, whereas glucocorticoid receptors (GRs) bind to glucocorticoids with a 10-fold lower affinity (Sapolsky et al., 2000). Both receptors are ligand-driven transcription factors, meaning that when unbound they primarily reside in the cytoplasm and after binding with cortisol (CORT) they migrate to the nucleus to directly and indirectly affect gene transcription (Groeneweg et al., 2011). These genomic effects comprise many common aspects of the stress response, but the specific genes affected by GRs and MRs are largely different (Datson, van der Perk, de Kloet, & Vreugdenhil, 2001).

We focus on GRs because their activation covaries with fluctuations in glucocorticoid levels (Stavreva et al., 2009), and while a few studies have investigated their impact on behaviour or immunity in wild animals (Landys, Piersma, Ramenofsky, & Wingfield, 2004; Landys, Ramenofsky, Guglielmo, & Wingfield, 2004; Lattin, Waldron-Francis, & Romero, 2013), it is not known whether they act as a mechanism of phenotypic integration of those traits in free-ranging animals. Experimental evidence suggests that although acute stress can downregulate GRs, bioavailability of GRs does not substantially vary over the course of a month under chronic stress (Paskitti, McCreary, & Herman, 2000). Furthermore, the developmental causes of variation in GR levels have been explored in detail, and indicate that differences in GR expression are stable into adulthood (Weaver et al., 2004). In turn, manipulating or blocking GRs should interfere with the effects that fluctuations in glucocorticoid levels (which change on the order of minutes, hours and days) produce via binding with the GR (Stavreva et al., 2009). This motivation is based on studies of rats (*Rattus norvegicus*) in laboratory conditions, but the evolutionarily conserved nature of the stress response supports applying it in other rodents (Ellis, Jackson, & Boyce, 2006). Manipulating a single pathway may shed light on the role that mechanisms play in consistent individual differences and correlations of behavioural and immune traits.

A wealth of research has elucidated relationships between glucocorticoids and behaviour (Cockrem, 2007; Downs et al., 2012; L. B. Martin et al., 2012; Reeder & Kramer, 2005) as well as glucocorticoids and immunity (Bourgeon & Raclot, 2006; Brooks & Mateo, 2013; Demas et al., 2011; Downs et al., 2012; L. B. Martin et al., 2012). Laboratory research on rodents has shown that acute and chronic elevation of glucocorticoids can also have differing effects on behaviour (Sapolsky et al., 2000). Likewise, in the short term, acute increases in glucocorticoids can help activate inflammation, but glucocorticoids are primarily anti-inflammatory, particularly when elevated chronically (Sorrells & Sapolsky, 2007). Long-term inflammation can produce glucocorticoid resistance (i.e. insensitivity), which can counter the dynamics described by Sorrells and Sapolsky (2007). Glucocorticoid resistance is often associated with major pathophysiology in humans (Gross, Lu, & Cidlowski, 2009; Pace, Hu, & Miller, 2007). It can also be caused by a week of social defeat in rats (Avitsur, Stark, Dhabhar, Padgett, & Sheridan, 2002). In wild alpine marmots, *Marmota marmota*, and eastern chipmunks, *Tamias striatus*, glucocorticoid measures positively correlate with open-field behaviour (Costantini et al., 2012; Ferrari et al., 2013; Montiglio, Garant, Pelletier, & Réale, 2012). These studies demonstrate that the stress response plays a key role in both behavioural and immune variation, suggesting that glucocorticoids can affect the relationship between behaviour and immunity. In essence, such an effect of glucocorticoids would be a three-way interaction among those factors, but this has rarely been tested in wild animals. L. B. Martin et al. (2012) found evidence of allocation trade-offs between flight performance and innate immunity in response to the stress of captivity in wild-caught house sparrows, *Passer domesticus*, but to our knowledge the effect of glucocorticoids on the relationship between behaviour and immunity has not been tested in a wild mammal, and no study on wild animals has evaluated the effect of glucocorticoids on multiple behaviours and immunity. Furthermore, GRs may play a key role in relationships between behaviour and immunity. However, few studies have directly addressed the impact of GRs on natural patterns of variation in either behaviour or immunity (e.g. Landys, Piersma, et al., 2004; Landys, Ramenofsky, et al., 2004; Lattin et al., 2013), with a particular lack of studies on the effect of GR–glucocorticoid binding on both behaviour and immunity to determine whether GR–glucocorticoid binding is responsible for relationships between those traits. The stress response's joint relationship with behaviour and immunity may be key to explaining the variation of each of those traits.

The stress response may cause behaviours and immunity to covary at multiple levels, both within and between individuals (Dingemanse & Dochetermann, 2013; Downs & Dochtermann, 2014). At one level, traits may covary within an individual as traits change together when individuals encounter differing conditions. At another level, the stress response may cause between-individual covariance, in which the individual average responses of two traits are correlated. Ferrari et al. (2013) found evidence that marmots' between-individual covariance among behavioural and physiological traits differed from the within-individual covariance pattern. Interestingly, glucocorticoids did not correlate with behaviour, heart rate or breathing rate at the between-individual level as predicted by the 'coping styles model', but rather showed correlations at the within-individual level in support of the recent 'two-axes model' (see Koolhaas et al., 2010 for details on models). In house mice, *Mus domesticus*, selected for high voluntary activity, corticosterone