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Wild state secrets: ultra-sensitive measurement of micro-movement can reveal internal processes in animals

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Assessment of animal internal "state" – which includes hormonal, disease, nutritional, and emotional states – is normally considered the province of laboratory work, since its determination in animals in the wild is considered more difficult. However, we show that accelerometers attached externally to animals as diverse as elephants, cockroaches, and humans display consistent signal differences in micro-movement that are indicative of internal state. Originally used to elucidate the behavior of wild animals, accelerometers also have great potential for highlighting animal actions, which are considered as responses stemming from the interplay between internal state and external environment. Advances in accelerometry may help wildlife managers understand how internal state is linked to behavior and movement, and thus clarify issues ranging from how animals cope with the presence of newly constructed roads to how diseased animals might change movement patterns and therefore modulate disease spread.

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Because an animal's use of space and its behavior will determine what it encounters (Lima and Zollner 1996), how it positions itself and behaves within its environment can offer valuable insight into its role in an ecosystem. This realization has driven the development of methods that define where wild animals occur within the environment (Hebblewhite and Haydon 2010), as well as approaches for determining animal behavior (eg Watanabe *et al.* 2005). Behavior is partly driven by internal "state" (eg Saarenmaa *et al.* 1988), which defines an animal's current status (Clark *et al.* 1997), incorporating components such as its nutritional, hormonal, immunological, and affective states (Mendl *et al.* 2011). The interaction between state and environment is important in driving behaviors (Duncan and Petherick 1991); accordingly, quantifying animal state should help us to understand strategies adopted by wild animals (Saarenmaa *et al.* 1988; Nathan *et al.* 2008). The internal state of an organism is best described by its internal conditions, the examination of which generally requires quantitative analysis of physiological variables such as blood chemistry, obtained through samples in laboratory or field settings (but see Signer *et al.* 2010).

Here we report on the potential of external, animal-attached tags containing accelerometers for use as novel and ultra-sensitive movement sensors, to provide field researchers with indicators of animal internal state. Development of this technology could allow scientists, resource managers, and policy makers to gain a more holistic understanding of why animals behave the way they do as they move through their environment.

In a nutshell:

- Recording devices attached to wild animals have already generated insights into animal ecology; accelerometers, for example, provide data that can help in determining behavior
- Accelerometry, which measures body micro-movements, has now been shown to indicate an animal's internal state; previously, this was possible only by means of laboratory tests
- Given that behavior is based on an animal's internal state and environment, the use of accelerometers should allow wildlife practitioners to better understand and predict behavior in wild animals

Determining state from posture and movement

Experimental set-up

To highlight the broad applicability of this approach, we examined three specific components of state in three different animal models: chemical state in humans (*Homo sapiens*), affective state in African elephants (*Loxodonta africana*), and disease state in death's head cockroaches (*Blaberus craniifer*). Small accelerometers (typical volume = ~10 mm^3) that quantify acceleration in three dimensions can be incorporated within animal-attached logging systems to record data that provide information on ani-

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mal posture and movement (Wilson *et al.* 2008). All subjects were equipped with tri-axial accelerometers, which were within full logging units taped to the wrists (humans, recording at 40 times per second [40 Hz]), attached in neck collars (elephants, recording at 320 Hz), or affixed to the body by copper wires to stream data to a computer (cockroaches, recording at 1000 Hz).

To study chemical state, we divided human trial participants into two groups: (1) users of the popular illicit drug, recreational ecstasy or MDMA ([±]3,4-methylenedioxymethamphetamine; Parrott 2013) but who were not under the influence of ecstasy at the time of the study and (2) subjects that had never used ecstasy. Members of both groups were asked to hold their arms out to their sides while data from wrist-attached accelerometers were recorded.

To investigate affective state, we observed elephant walking behavior, and this was classified as either (1) "positive" when the subject was walking between two of three desired resources (food pile, mud wallow, or dust bathing area), without a dominance interaction, or (2) "negative" when the subject was walking away after being displaced by the socially dominant animal of the herd. "Positive walking" could represent motivation or anticipation (generally a positive affective state) whereas "negative walking" could mirror fear or anxiety (a negative affective state).

To examine the manifestation of a disease state, we compared a group of healthy adult *B craniifer* with a second group we infected with *Metarhizium anisopliae*, a fungus that attacks insects (Butt *et al.* 1994). Members of both cockroach groups were stimulated daily to elicit an escape response, causing them to run up a 2-m trough while data were recorded via dorsally (pronotum)-attached accelerometers.

We transformed the accelerometry data to derive metrics that could be used to help define internal state, following the general behavioral approach described in Shepard *et al.* (2008), which advocates separating each of the three acceleration channels into static and dynamic components. The static component is a running mean of the raw data (here averaging over 2 s for the vertebrates and 20 ms for the cockroaches) and is useful for identifying posture. The dynamic component is essentially the "bounce" in the way an animal moves and is derived by subtracting the running mean values (see above) from the raw data in each channel. To derive an overall dynamic acceleration value, we summed the dynamic acceleration values of all three axes vectorially to give the vectorial dynamic acceleration (VDA; Shepard *et al.* 2008). We also used customized software (GRAPPLER; compare with Grundy *et al.* 2009) that is designed to allow multiple channels derived from acceleration data to be visualized in various ways, including by color coding; this visualization facilitated signal processing by highlighting patterns that might otherwise not have been apparent.

Results

In each of the three studies, there were statistically significant differences in accelerometer data ($P < 0.05$) that accompanied states and state transitions. We acknowledge, however, that statistical significance does not necessarily indicate the biological importance of the states and that continued development of assays is required for more comprehensive studies. We noted, however, that different features of the data were indicative of state for each of our case studies.

When looking at chemical state in humans, marked differences in movement – specifically "tremor" values (VDA; Shepard *et al.* 2008) – were observed when the subject's arms were held out horizontally, with drug users showing statistically higher VDA scores at each timepoint (Figure 1). For affective state in elephants, the three axes of the static acceleration showed that body posture varied according to the animals' determined affective state (positive or negative; Figure 2). For disease state in cockroaches, significantly decreasing VDA values over time were associated only with the infected animals so that the "dynamism" in each stride decreased with progressing fungal infection (Figure 3).

Links between acceleration and state

Accelerometers in animal-attached data loggers are becoming standard tools for identifying animal behaviors (Yang and Hsu 2010; Brown *et al.* 2013), given that they are able to quantify movement in a manner that cannot be achieved by unaided human observers, even when direct observation is possible. For example, Halsey *et al.* (2009) not only measured the frequency of wing beats of raptors wearing accelerometers, which is possible by direct observation, but also derived metrics for energetic effort. The value of accelerometers for defining movement, including micro-movement (which we define here as any movement that cannot be seen well enough to be quantified with the naked eye), stems from the physical measurement of a fundamental metric (acceleration) with high accuracy and with no element of subjectivity. Accelerometers such as the ones used in this study are able to resolve acceleration to within 0.004 g up to 1000 times per second per channel; advances in technology will enhance this further. Due to this sensitivity, researchers are now able to examine body micro-movements and determine how they relate to state. Flavel *et al.* (2012), for instance, reported how digit tremor in humans is symptomatic of ecstasy/MDMA use. Likewise, accelerometers appropriately placed on the skin can monitor a variety of body micro-movements, such as heart beating, which makes the body pulse (compare with Wilson *et al.* 2004). Thus, we predict that studies using accelerometers to document body micro-movements will become more common as increasingly sensitive accelerometers and related analytic software become available.

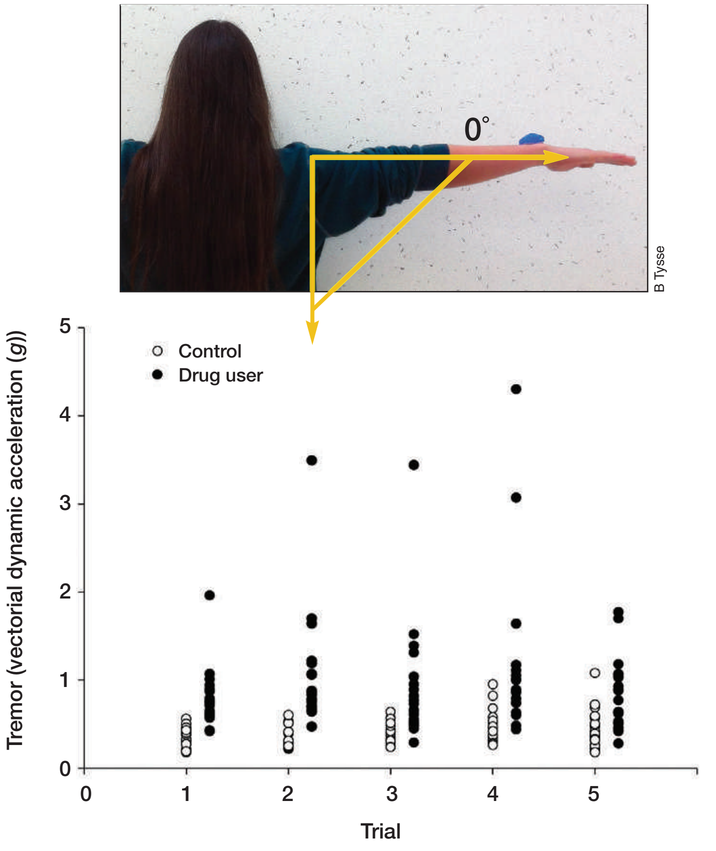

Figure 1. *Human tremor (measured as vectorial dynamic acceleration [VDA], where higher scores indicate more movement) measured at the wrist in a total of 40 people. Each subject completed five different tasks involving holding the arms as stable as possible in different positions (1 = participants seated, with arms held forward, horizontal for 60 s; 2 = as 1 but with arms held to the side; 3 = as 1 but standing; 4 = as 2 but standing; 5 = as 1 but with the dominant hand extended only, holding a pen between index finger and thumb). Circles indicate individual mean VDA over 10 s per task for 20 drug users [having used stimulant drugs regularly for at least 2 years] (solid circles) and 20 non-drug users (open circles). Significant differences between groups were observed for each of the five trials (Mann-Whitney* U*; P < 0.01).*

A critical question regarding the usefulness of this tool is the extent to which accelerometer signals can code exclusively for varying states, given that interpretation of behavior and state are often context dependent – and that context may not be known. This question will be answered only with further investigation but the diversity of the signals produced by accelerometers should be useful. Modern accelerometers measure in three dimensions at rates of up to many hundreds of times per second, which helps define repetitive waveforms (eg Figure 3). Each channel nominally records the sum of the static (gravity-based) and dynamic (animal movement-based) components. These components can be deconstructed and each signal from each channel can be used to calculate additional derivatives. Any combination of these measures can be examined for variability within and between waveforms in a manner analogous to, for example, human studies investigating heart rate variability according to lifestyle (Thayer *et al.* 2010). Accelerometers can be subjected to almost infinite variability by being placed on different parts of the body. Thus, although tri-axial accelerometers only nominally measure in three axes, the combination of factors can produce considerable variation, just as the strings on a violin can produce many different musical sounds.

The reasons why body micro-movements might change with internal state are complex and will likely depend on the specific state. Although not intentionally conducted to examine the mechanisms behind altered movements, Parrott (2013) reported chemical-state-related movement problems in MDMA users and suggested that MDMA-induced serotonin (5-ht) toxicity produces motor system dysfunction. A similar issue has been reported for cocaine users (Wilcox and Wilcox 2009) and is attributed to excessive dopamine from cocaine consumption. More recently, Flavel *et al.* (2012) used accelerometers to examine finger tremor variation at rest and during movement between ecstasy/MDMA users, amphetamine users, and cannabis users (though the subjects were not under the influence of the drugs at the time of the study), and non-users; however, only the MDMA users and controls differed in the amounts of "tremor" seen during movement tasks. Similarly, the differing patterns of arm tremors between ecstasy users and non-users in our study may be generally explained by motor disorders, pointing to a movement-based manifestation of abnormal "physical state" in drug-using populations, although the precise mechanisms are unclear. Careful examination of micro-movement characteristics in non-clinical participants might therefore be useful as early markers for movement disorders. In wildlife, ingestion of certain plant-based foods, particularly those with phytochemical toxins intended to deter consumption (Hoy *et al.* 1998), may result in analogous micro-movement deviations from the norm, potentially providing ecologists with equivalent metrics.

Manifestation of disease state will span conditions ranging from neurological disorders – such as Parkinson's disease and bovine spongiform encephalopathy, where atypical movement should be readily apparent using accelerometry – to illnesses that are not specifically muscular or neurological in effect. In the latter cases, however, diseases also lead to generally compromised performance and decreased energy levels, which we would expect to be reflected in changes to both postural and dynamic movements. Certainly, the cockroaches in our study exhibited both postural changes and reductions in VDA (Figure 3), so this general framework would seem a reasonable way to examine animal health.

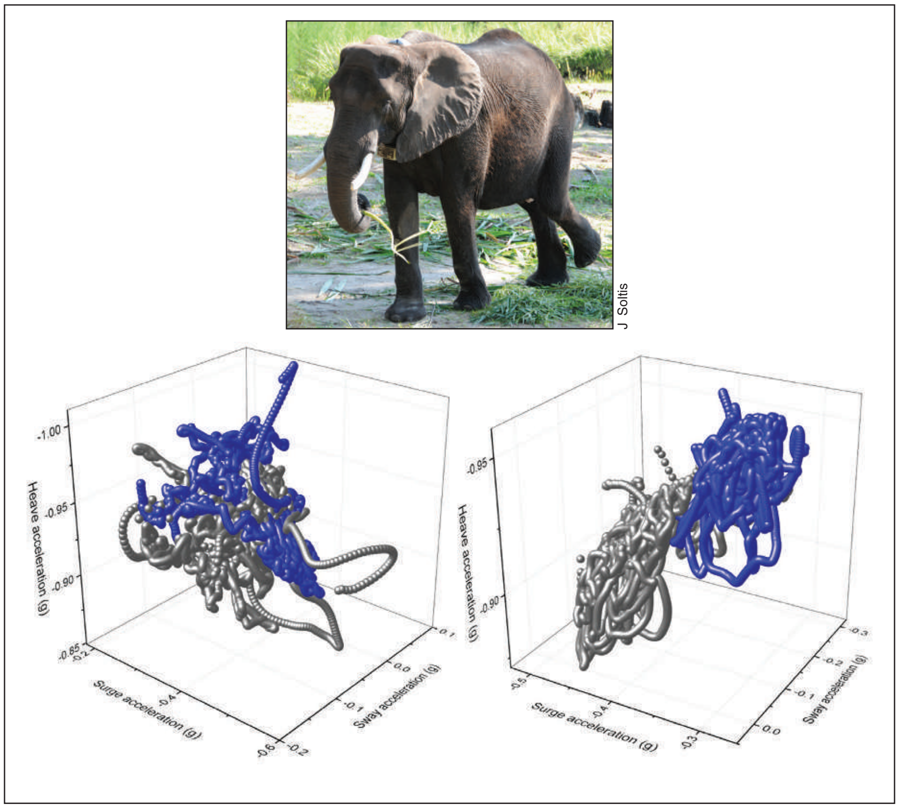

Figure 2. *African elephant (*Loxodonta africana*) posture derived from two adult females, represented by running mean (over 2-s) values of acceleration measured in the vertical, lateral, and anterio-posterior axes of the animals. In the figure, data from multiple separate periods are combined according to the animals' perceived affective state (positive: blue points; negative: silver points). The two states occupied significantly different probability distributions in three-dimensional space: the statistical process (i) removed the autocorrelation structure, (ii) found the residuals of the positive walking, and (iii) modeled the negative walking that was closest to the positive before (iv) obtaining a forecasting error for the positive walking series and (v) testing the differences using a Kolmogorov-Smirnov test (using threshold values of* $P < 0.01$*).*

Descriptions of animal health usually include documentation of hormone concentrations, particularly where stress hormone levels are undesirably high (Lundberg 2005). The effect of large amounts of stress hormones is an increased metabolic rate coupled with enhanced "vibrancy" (ie tremors; Lazarus and Folkman 1984). This effect, similar to the tremors observed in ecstasy users, should be detectable via accelerometry, albeit possibly at a different frequency. Hormonal state also drives affective state to an extent, and since ecstasy users have cortisol levels that are 400% higher than controls (Parrott 2013), this may contribute to their psychomotor problems. We note, however, that the two broad affective states proposed in the elephant study (Figure 2) presumably have a neurological basis rather than a hormonal one. The possibility that we may be able to allude to animal mental well-being, even if couched in terms such as "affective state", has broad ramifications for wild and domestic animal welfare. Within human society we are generally aware of the emotional state of others by the way they walk (eg www.biomotionlab.ca/Demos/BMLwalker.html), as posture and dynamism in the gait show a person's condition. Given that there is an optimum way to walk (Sekiya *et al.* 1997), the advantages in deviating from this to a manifestation of state are not clear. Presumably, state manifestation through gait confers the greatest advantage in social animals, and for this reason may be less obvious in solitary species. On the other hand, where state indicates condition in within-species competitive interactions such as male–male duels, we might expect selection pressure for reduced signs of "weakness" (and a correspondingly enhanced ability to discriminate an opponent's state). Similarly, it has been observed that predators can be highly discriminatory in their selection of prey, based on the way they move (Mills 1990).

Implications for wildlife practitioners

There are two levels at which the study of internal state may prove helpful for wildlife practitioners, managers, and policy makers. The first level involves the state of a select number of tagged wild animals that are subject to changing conditions (known or proposed), such as oil pipeline construction in Alaska or road construction in Tanzania (Dobson *et al.* 2010). Here, accelerometers could provide a seamless record of how animals exposed to the possible stressor react, both behaviorally and with respect to their "state" as indicated by micro-movements. Importantly, however, where the stressor leads to increased stress hormone levels but does not elicit an apparent change in behavior (Kiank *et al.* 2010), examination of micro-movements may still help to identify the condition of stress. Research is clearly needed to demonstrate a specific link between stress hormone levels and accelerometry signatures. The second level is more expansive. Larger numbers of accelerometer-equipped animals within the environment for general monitoring, or as part of a project with a different primary focus, should provide adequate information to examine a suite of important issues ranging from animals' energy reserves (well-fed and malnourished animals move differently; Wilson *et al.* 2006) to animal stress and health. Comparison of states among animals from different areas within the environment could help not only in compar-

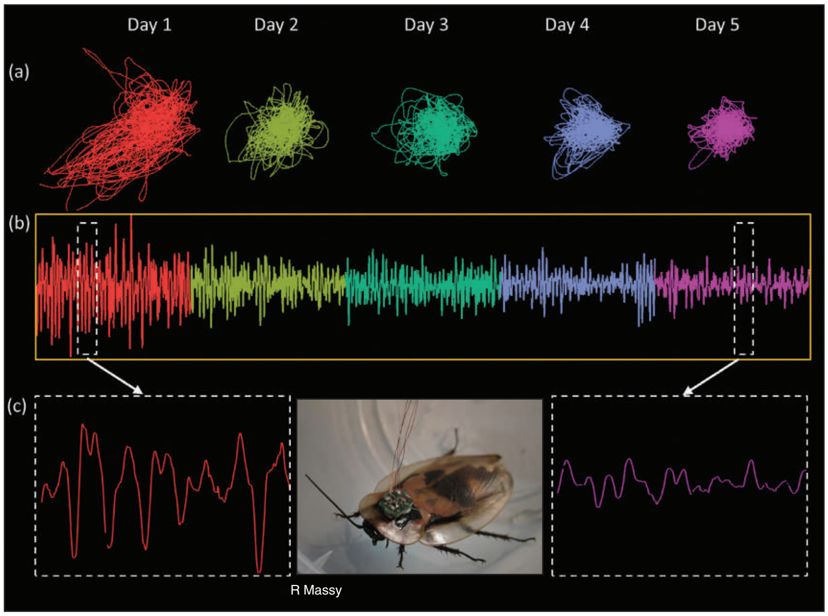

Figure 3. *Comparison of four fungus-infected and four non-infected cockroaches* (Blaberus craniifer) *showed that vectorial dynamic acceleration (VDA) decreased over time in the infected animals and increased over time in non-infected animals in a significant interaction (GLMM (lmer(VeDA~DAY*TREATMENT+(1|ID))) with individual as a random effect [interaction effect $\chi^2 = 19.245$, degrees of freedom = 1, $P < 0.001$]). The GRAPPLER visualization of an infected animal shows each day after being infected as a different color and gives (a) heave versus sway acceleration plots, (b) heave versus 2-s time plots, and (c) expanded time inserts of the heave versus time plots to depict how footfalls, visualized in waveforms, differed between day one and day five of the infection.*

ing their area- and site-dependent well-being but also in elucidating the dynamics of disease spread. This latter issue is notoriously problematic in modeling best strategies for managers (Gilbert *et al.* 2010). Indeed, the diagnosis of subclinical illnesses, such as bovine spongiform encephalopathy, which is particularly difficult to establish (Atarashi *et al.* 2011), may be facilitated by micro-movement studies. Beyond that, given that state is considered one of the fundamental drivers of animal movement (Nathan *et al.* 2008), the definition of state, coupled with examination of how it relates to observed movement patterns, might provide analysts with the hitherto unrealized ability to understand the dynamics of disease spread.

It may transpire that examination of micro-movement may offer little practical value to defining the specifics of animal state. This would be disappointing, particularly after the necessary careful and appropriate ethical and experimental caveats have been taken into account to minimize negative handling effects and ensure best practices. However, finding no practical value in a determination of state based on micro-movement seems unlikely given the complexity and variety of accelerometry signals. Yet this very complexity, in the form of idiosyncratic, species-specific, and state-specific variation in accelerometry signals, will require careful observation and experimentation. If state can indeed be defined from acceleration data, even at a baseline level for comparative purposes, practitioners may be able to use it to be alerted to unwanted ecosystem changes that could be prevented by rapid examination of causes and treatments. Nevertheless, researchers must be mindful of how potential gains in understanding animal state by means of animal-attached accelerometers are measured against possible detriment to animal test subjects; to that end, properly devised studies will be critical.

Accelerometers show real potential for helping to discern internal state in animals in both field and captive settings. This preliminary study has obvious caveats, not least of which is the uncertainty involved in determining the point at which a micro-movement becomes a behavior, although the ability of the accelerometers to distinguish between these is subject to debate. Such caveats will need to be adequately resolved. Nevertheless, the breadth of topical issues where definition of state would markedly enhance our understanding of processes is wide, making realization of this exciting new approach a boon for wildlife practitioners.

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