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Behavioral implications of rapid changes in steroid production and action in the brain

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Not so long ago, most behavioral neuroendocrinologists probably believed that the steps of steroid action located up-stream from the binding of the hormone to its intracellular receptor had been largely elucidated so that research could now concentrate on the identification of genes transcribed under the control of steroids and their role in cellular function including activation of behavior. During the last 10–15 years, several important discoveries have clearly forced us to re-evaluate this notion. For example, a second nuclear estrogen receptor was identified first in humans (Kuiper et al., 1996) and then in a variety of other vertebrates (e.g., Foidart et al., 1999; Mosselman et al., 1996; Tremblay et al., 1997). A new family of proteins regulating steroid-dependent gene transcription, the steroid receptor co-regulators, was also discovered and now includes approximately 300 members (McKenna et al., 1999; Tetel et al., 2009).

More relevant to the present topic, it was also progressively realized that sex steroids can be completely synthesized in the brain from cholesterol (Baulieu et al., 1999; Do Rego et al., 2009). The functional significance of this synthesis especially for the control of behavior remains, however, largely unclear to this date, with only a few exceptions (e.g., Soma et al., 2008). Additionally, it was shown that the activity of brain aromatase, the enzyme that produces estrogens by aromatization of androgens such as testosterone can be modulated within minutes, and possibly more rapidly, by calcium-dependent phosphorylations of the enzymatic protein (Balthazart et al., 2003; Balthazart et al., 2006b). Interestingly these fast enzymatic changes can be controlled by variations in neurotransmitter activity (in particular glutamatergic inputs; Balthazart et al., 2006a; Remage-Healey et al., 2008) and recent work demonstrates that they actually result in measurable variations in brain estrogen concentrations (Remage-Healey et al., 2008) that have significant effects on behavior and brain auditory physiology (Balthazart et al., 2006b; Remage-Healey and Bass, 2004; Tremere et al., 2009).

On another related front, it was progressively realized that steroids, estrogens in particular, have rapid effects in the brain mediated by their action at the level of the cell membrane which then modulates a variety of intracellular signaling cascades. It had been known for quite some time that estrogens affect gonadotropin-releasing hormone neurons very rapidly with latencies that are clearly incompatible with effects mediated through changes in transcription (Kelly et al., 1976) and similar effects were over the time demonstrated in various cellular systems (e.g., Kelly et al., 2003; McEwen and Alves, 1999; Vasudevan and Pfaff, 2008; Woolley, 2007).

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During the past ten years, data have slowly accumulated indicating that rapid effects of estrogens in the brain are highly relevant for the control of sexual and aggressive behaviors. It was originally shown that ano-genital investigations and mounting of the female were stimulated in male rats within 35 min following a single injection of estradiol (Cross and Roselli, 1999). Comparable rapid effects of estrogens on reproductive behaviors were then identified in various classes of vertebrates including fishes (Remage-Healey and Bass, 2004), birds (Cornil et al., 2006) and mammals (Kow and Pfaff, 2004; Taziaux et al., 2007; Trainor et al., 2007; Trainor et al., 2008) suggesting that this phenomenon is widespread and phylogenetically ancient.

Taken together these data indicate that estradiol displays a number of features similar to those of a neurotransmitter (production in the brain at the presynaptic level, rapid changes in local concentrations and fast action on brain physiology and behavior; Balthazart and Ball, 2006; Cornil, 2009; Cornil et al., 2006). This conclusion is, however, not necessarily specific to estradiol as indicated by a paper of Pradhan and collaborators published in this issue of *Hormones and Behavior* (Pradhan et al., 2010). This paper touches on two of these new aspects of steroid physiology in the brain: their local synthesis and the socially-driven fast changes in their production.

It has been known for quite a while that territorial aggression is maintained in song sparrows outside the breeding season while testes are regressed and plasma testosterone concentrations are essentially undetectable (Wingfield and Hahn, 1994). Therefore this aggression probably does not depend on testicular testosterone for its activation and accordingly, castration does not decrease the expression of the behavior (Wingfield and Hahn, 1994). Soma and collaborators then showed that significant concentrations of the androgen precursor, dehydroepiandrosterone (DHEA) remain present in the blood of these birds during the fall (Soma and Wingfield, 2001). These data gave rise to the idea that this steroid might serve as a substrate to produce sex steroids in the brain, such as testosterone or its metabolite estradiol, that could then activate aggressive behavior. This idea was supported by the observation that pharmacological blockade of sex steroid action (by injection of an antiandrogen) and production (by aromatase inhibition) inhibits aggression outside the breeding season (Soma et al., 1999; Soma et al., 2000), indicating that this behavior remains steroid-dependent even in the absence of substantial concentrations of testosterone in the plasma. Consistent with this notion, the enzyme 3β -hydroxysteroid dehydrogenase/ $\Delta 5$ - $\Delta 4$ isomerase (3β -HSD), the key limiting enzyme allowing transformation of DHEA into sex steroids (androstenedione, testosterone and estrogens) is present in the brain (Soma et al., 2004) but the mechanisms controlling its expression and activity are still poorly understood. It was, however, shown recently that the activity of this enzyme is rapidly inhibited by restraint stress (Soma et al., 2004) and by estradiol (Pradhan et al. 2008) indicating that brain production of androgens can vary in a rapid manner as previously shown for estrogens but the functional significance of these changes was not identified.

In the present paper, new data are reported demonstrating that brain 3β -HSD activity is regulated in an anatomically specific manner both in the long term (between seasons) and acutely, as a result of behavioral interactions. The authors first showed that 3β -HSD activity is significantly higher during the fall than in the reproductive season in the diencephalon and in the central, caudal and ventromedial parts of the telencephalon but not in other brain areas such as the optic lobes or rostral telencephalon. At the end of the breeding season, there is thus in several parts of the brain an up-regulation of the enzymatic activity that is able to produce increasing amounts of androstenedione and testosterone from the precursor DHEA which still circulates at high concentrations in the blood. The specific mechanisms regulating this seasonal change in 3β -HSD activity have not been identified but interestingly they occur at a time when peripheral concentrations testosterone and presumably central concentrations of its estrogenic

metabolites are declining. Considering that estradiol acutely (within 10 min) inhibits 3 β -HSD activity in zebra finches (Pradhan et al., 2008), it is tempting to speculate that this control mechanism, identified so far in acute mode, might also play a more chronic role and control of seasonal changes in 3 β -HSD activity. The locally produced androgens should be available to activate aggressive behavior, which then depends on steroids produced in the brain rather than in the testes (see Figure 1). This explains why castration has no effect on this behavior that remains nevertheless steroid-dependent and is inhibited by pharmacological agents blocking androgen action or their aromatization into an estrogen (Soma et al., 1999; Soma et al., 2000).

More importantly, the present studies demonstrate that this central production of behaviorally active sex steroids is acutely regulated during social interactions. During a second experiment, the authors exposed male song sparrows to either simulated territorial intrusions (introduction of a live caged conspecific decoy and of conspecific song playback in the center of the territory) or an empty cage without playback (control condition) for 30 min during the fall. 3 β -HSD activity was measured in their brain immediately after the test. Engaging in aggressive interactions with this simulated "territorial intruder" rapidly increased neural 3 β -HSD activity, again in a region-specific manner. This increase was highest in the central medial telencephalon (515%) a region that contains the septum (a brain area implicated in aggression control; e.g. Goodson, 1998) but also present in the caudal telencephalon (242%), the region including the arcopallium (equivalent to parts of the mammalian amygdala) and in particular the robust nucleus of the arcopallium, a part of the song control circuit. No effect was observed in the rostral telencephalon. Interestingly, 3 β -HSD activity was positively correlated with the amount of time that subjects spent in close proximity (less than 1 m) to the intruder decoy, which reflects the intensity of the aggressive behavior displayed by the territory owner. This correlation therefore supports the notion that the change in enzymatic activity specifically relates to the behavioral interactions.

It is also critical to note that the increase in 3 β -HSD activity induced by the simulated intrusion was only observed when activity was measured without addition of exogenous NAD⁺ (nicotinamide adenine dinucleotide), the required cofactor of the enzyme. When standard amounts of this cofactor were added in the assay tubes, no difference in 3 β -HSD activity was detected between control birds and birds exposed to the simulated territorial intrusion (except in the cerebellum where STI decreased enzymatic activity). This suggests that the acute regulation by aggression of the 3 β -HSD activity may not be the result of a direct effect on the enzyme but rather an indirect consequence of changes in the local availability of a critical co-factor. An interesting alternative interpretation raised by the authors is that in the absence of exogenous NAD⁺, the *in vitro* assay specifically measures a pool of enzyme located in a NAD-rich cellular compartment, such as mitochondria. Additional work will be needed to discriminate between these possibilities.

Finally, although the absolute amounts of estrogenic metabolites (estrone + estradiol) was not affected following simulated territorial intrusions, their contribution to the total amount of 3 β -HSD metabolites produced from tritiated DHEA was significantly decreased in the central medial and caudal telencephalon. This decrease was observed despite the fact that the production of the aromatizable androgen androstenedione increased in these conditions. Because this substrate concentration increased and it was presumably a limiting factor for the aromatization reaction (its concentration in the brain is apparently much lower than the *K_m* of the enzyme), these data suggest that territorial intrusions and the ensuing aggressive behavior inhibit aromatase activity in sparrows within a similar time frame as performance of sexual behavior does in quail (Cornil et al., 2005).

These results are important in that they provide a new model for the regulation of aggression (and possibly behavior in general) based on androgens produced in the brain by local metabolism of a behaviorally inactive circulating precursor. Furthermore they demonstrate that this brain production of behaviorally active steroids is acutely regulated by the social environment and/or the behavioral activity of the subjects so that a fine grain temporal adaptation of the steroid-dependent controls can potentially be achieved in an anatomically specific manner. Similar conclusions concerning estradiol had previously been reached in several species ranging from fishes to mammals based on different types of experimental designs and results. Rapid changes in the production and action of behaviorally active steroids thus seem to represent a fairly widespread phenomenon and its relative importance in comparison with the more classical genomic mode of action of steroids should be systematically investigated. This finding obviously opens a large number of questions regarding the mechanisms that regulate these enzymatic activities and the specific mode of action of steroids on neuronal physiology that mediates the activation of behaviors.

Concerning the regulation of steroid metabolizing enzymes in the brain, there is now a substantial corpus of data indicating that the activity of key steroidogenic enzymes in the brain is controlled by neurotransmitters (Do Rego et al., 2009). This provides mechanisms for the fine adjustment of steroid production and action by the endocrine state of the animal and by the inputs reaching its brain (signal from the environment, from congeners, from sexual partners...). The details of the cellular signaling cascades that mediate the action of neurotransmitters on enzymatic activities remain, however, unknown. We showed that brain aromatase activity can be rapidly modulated by phosphorylation processes (Balthazart et al., 2003). The present study suggests that the control of co-factor concentrations (NAD⁺ for 3 β -HSD, NADPH [nicotinamide adenine dinucleotide phosphate] for aromatase) could represent an alternative mechanism. The detail of these cellular mechanisms will now need to be identified.

This will, however, still leave open the big question of the cellular mechanisms that mediate the rapid actions of steroids on behavior. These effects are probably too fast to be mediated via changes in genomic transcription. Rapid non-genomic effects of steroids in the brain were first identified at the cellular level more than 30 years ago when Martin Kelly and Bob Moss identified the rapid regulation of the gonadotropin releasing hormone neurons by estradiol (Kelly et al., 1976; Kelly and Ronnekleiv, 2002). Since this early work, a large number of rapid effects of sex steroids, most of which occur at the level of the neuronal membrane, have been identified and have been shown to modulate a variety of intracellular signaling cascades (e.g. changes in intracellular calcium concentrations, interaction with G protein-coupled receptors, with neurotransmitter action, etc). However, with a few exceptions (e.g., Kow and Pfaff, 2004; Micevych and Dominguez, 2009; Tremere et al., 2009) we do not know what mechanisms play a role in the control of behavior. Their identification probably represents one of the next frontiers in behavioral neuroendocrinology.

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Fig. 1.

Aggressive behavior is displayed almost continuously throughout the year in song sparrows. During the spring, this behavior is activated by testosterone (T) originating from the testes. In the fall, this testicular production of testosterone is low to undetectable, but dehydroepiandrosterone (DHEA) of adrenal origin still circulates in significant concentrations. Thanks to an increased activity of the 3β -hydroxysteroid dehydrogenase/ $\Delta 5$ - $\Delta 4$ isomerase (3β -HSD), DHEA can be transformed in the brain into androstenedione and T and subsequently into estrone and estradiol (E_2) that activate fall aggression. The 3β -HSD and aromatase activities are also acutely regulated by the aggressive interactions (up- and down-regulation respectively). This socially driven change in brain steroid metabolism is demonstrated by

Pradhan et al (2010) to occur in the fall but future research should investigate whether a similar effect also takes place in the spring The size of letters representing steroids or enzymes in the figure is proportional to their concentration or activity