Journal Club

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# A Balancing Act: $D_4$ Receptor Activation and the Neurobiological Basis of Emotional Learning

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Review of Lauzon et al.

The neural mechanisms by which emotionally significant memories are encoded and recalled, and their involvement in normal and disordered brain function, remain elusive. Glutamatergic projections from the basolateral amygdala (BLA) to the medial prefrontal cortex (mPFC) are critically implicated in both the acquisition and extinction of conditioned fear (Maren and Quirk, 2004). Dopaminergic efferents from the ventral tegmental area overlap with inputs from the BLA in the mPFC (Pinto and Sesack, 1999 in Floresco and Tse, 2007). These mesocortical dopaminergic inputs are particularly responsive to aversive situations such as fear conditioning (Yoshioka et al., 1996) and play an important role in the modulation of BLA-evoked changes in mPFC neuronal activity (Floresco and Tse, 2007). Dysregulation of these neural circuits is thought to underlie emotional and cognitive disturbances in diseases such as schizophrenia, depression, and drug addiction (Floresco and Tse, 2007). Elucidation of the mechanisms by which the mPFC processes associative information and emotional memory at the cellular level and in the context of behavior will offer important new insight into such disorders.

Lauzon et al. (2009) have recently demonstrated a key role for dopamine in the regulation of BLA–mPFC processing of fear-associated memories. Using an olfactory fear conditioning assay in rats, these researchers selectively manipulated $D_1$ and $D_4$ dopamine receptor subtypes ($D_1$R and $D_4$R) shortly before either conditioning (acquisition phase) or testing (challenge phase). Their work has demonstrated that bilateral activation of $D_4$Rs in the mPFC with a highly selective $D_4$R agonist immediately before the presentation of a mild, emotionally nonsalient footshock elicited a dose-dependent potentiation of associative fear conditioning. Yet, preconditioning $D_4$R activation with the highest dose of this agonist blocked the acquisition of this cue-associated learning for a stronger, emotionally salient footshock. The selectivity of the $D_4$R-mediated facilitation of cue-associated learning for the nonsalient footshock was confirmed with coinfusion of a competitive $D_4$R antagonist with the selective $D_4$R agonist. Conversely, $D_4$R activation before the testing phase did not significantly affect expression of the previously acquired conditioned response. These findings suggest that $D_4$R activation is necessary for the encoding, but not the retrieval, of a conditioned fear memory. Alternatively, the expression, but not the acquisition, of an emotionally pertinent memory (salient footshock) was selectively blocked by pharmacological activation of $D_1$Rs with a selective $D_1$R agonist.

$D_4$R-mediated potentiation of emotionally nonsalient associative fear conditioning was dependent on a functional connection between the BLA and mPFC (Lauzon et al., 2009). Although present at low levels on mPFC pyramidal neurons, $D_4$Rs are predominantly expressed in GABAergic interneurons that receive BLA input (Mrzljak et al., 1996; Gabbott et al., 2006). Pharmacological activation of mPFC $D_4$Rs decreases BLA-mediated activation of inhibitory neurons in the mPFC and may thereby serve to prime pyramidal neurons to receive inputs relevant to emotionally salient associations (Floresco and Tse, 2007). Laviolette et al. (2005) have demonstrated that a subpopulation of mPFC pyramidal neurons receiving inputs from the BLA displays strong associative increases in neuronal activity when the animal is exposed to odors predictive of footshock. The encoding of these neuronal responses and the expression of conditioned fear were blocked by inhibition of mPFC $D_4$Rs before conditioning. Together, the findings of Lauzon et al. (2009) and of Laviolette et al. (2005) demonstrate that mPFC $D_4$Rs and BLA afferents critically mediate fear-associative learning.

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Other recent work has also demonstrated that $D_4R$ activation is a key molecular mechanism mediating neuronal plasticity in the PFC. Yuen and Yan (2009) have shown that $D_4R$ activation either by an exogenous agonist or by endogenous dopamine suppresses AMPA receptor-mediated synaptic transmission in PFC GABAergic interneurons through calcium-dependent actin/myosin-mediated regulation of AMPA receptor trafficking. $D_4R$ actions also mediate downregulation of $GABA_A$ or NMDA receptor expression in PFC pyramidal cells via actin/myosin-dependent (Graziane et al., 2009) or $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII)-dependent (Wang et al., 2003) mechanisms, respectively. Thus, mediation of synaptic plasticity and network integrity through $D_4R$ regulation of receptor trafficking in mPFC GABAergic interneurons and pyramidal cells activated by BLA inputs may, at least in part, regulate emotionally salient cue-associated learning.

A critical level of $D_4R$ activation, however, appears necessary to fine-tune BLA-mediated encoding in the mPFC. In this regard, $D_4R$ agonist treatment before conditioning augmented the encoding of mild, nonsalient footshock associations yet blocked learning of fear associations paired with stronger, salient stimuli (Lauzon et al., 2009). This blockade of conditioned fear associations is similar to that observed with intra-mPFC preconditioning application of a selective antagonist that prevented endogenous dopamine activation of the $D_4R$ (Laviolette et al., 2005). Given that stressful and aversive stimuli strongly induce the release of dopamine in the mPFC and that such release is associated with conditioned fear learning (Yoshioka et al., 1996), it is possible that agonist supplementation of normally sufficient endogenous $D_4R$ stimulation in the study by Lauzon et al. (2009) served to disrupt normal associative learning processes to emotionally salient stimuli. This suggests that suboptimal or supranormal $D_4R$ stimulation in the mPFC impairs the encoding of emotional memory (Fig. 1), similar to that reported for the relative effects of PFC $D_1R$ stimulation on working memory (Zahrt et al., 1997).

The mechanism(s) by which neuroplasticity is modulated by this inverted U-shaped dose–response curve for dopamine receptor activation (Zahrt et al., 1997; Monte-Silva et al., 2009) to regulate associative learning warrants further consideration. A reduction of GABAergic feedforward inhibition (Floresco and Tse, 2007) and downregulation of AMPA and $GABA_A$ receptor expression in GABAergic and pyramidal cells, respectively (Graziane et al., 2009; Yuen and Yan, 2009), may contribute to facilitating emotionally salient associative learning when levels of $D_4R$ activation are optimal. This could explain the ability of the $D_4R$ agonist to augment cue-associated learning for nonsalient stimuli that do not elicit sufficient dopamine release on their own (Lauzon et al., 2009). Conversely, blockade of endogenous dopamine activation of the $D_4R$ would prevent this $D_4R$-mediated disinhibition and receptor trafficking, and in turn, the learning of cues associated with the salient stimulus (Laviolette et al., 2005). However, the mechanism by which supranormal $D_4R$ stimulation induced by endogenous plus pharmacological $D_4R$ activation inhibits associative learning is less clear. If this optimal $D_4R$ response indeed involves downregulation of AMPA and $GABA_A$ receptor expression in mPFC GABAergic and pyramidal cells, respectively (Graziane et al., 2009; Yuen and Yan, 2009), supranormal stimulation may prevent activation of these calcium-dependent actin/myosin-mediated mechanisms.

Lauzon et al. (2009) speculate that mPFC $D_4R$-mediated biphasic regulation of CaMKII activity may account for the differential effects of the $D_4R$ agonist in mediating the acquisition of associative fear memories during subthreshold and suprathreshold footshock conditioning. They suggest that low versus high levels of mPFC neuronal activity may have resulted from the nonsalient and salient footshock conditions, respectively, such that $D_4R$ activation would correspondingly enhance or inhibit CaMKII activity (Gu and Yan, 2004). However, findings of Laviolette et al. (2005) demonstrated that $D_4R$ antagonist treatment blocked BLA-mediated associative learning in response to the same salient footshock, suggesting that the level of mPFC neuronal activity was not the mediating factor. Instead, we propose that $D_4R$ activation by what was considered to be the effective $D_4R$ agonist dose, in addition to the corelease of dopamine in the mPFC *in vivo*, may have tipped the facilitory actions of these receptors to an inhibitory action with respect to effects on the encoding of associative fear memories, possibly via a calcium-dependent mechanism. Whether this involves CaMKII remains to be determined. Unfortunately, the dose–response relationship established for the nonsalient fear conditioning was not conducted for the salient response. This would have provided some insight into the validity of our proposed mechanism.

The appropriate level of $D_4R$ activity required within the mPFC to enable the

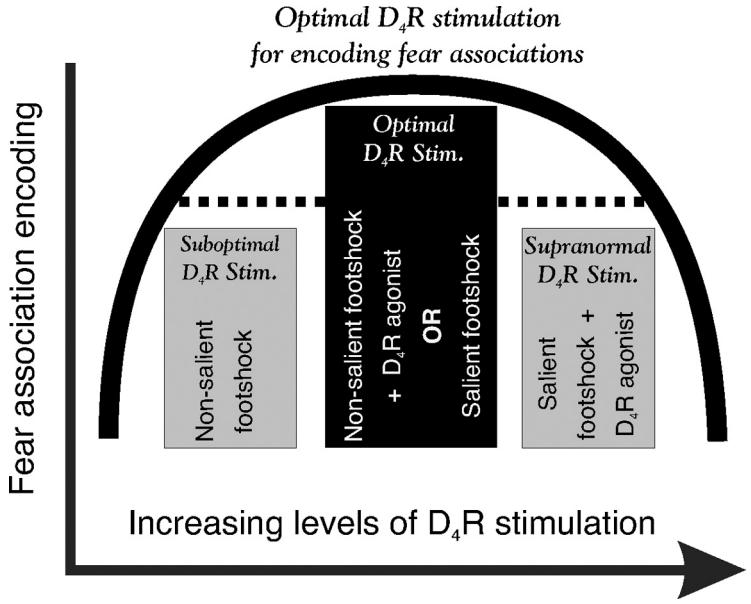


**Figure 1.** Hypothesized inverted U-shaped $D_4R$ response curve illustrating that either suboptimal or supranormal $D_4R$ stimulation could impair encoding of conditioned fear associations. The suboptimal condition represents insufficient endogenous dopamine stimulation of $D_4Rs$ during subthreshold nonsalient footshock. The supranormal condition represents excessive $D_4R$ stimulation from the combined actions of an intra-mPFC $D_4R$ agonist and endogenous dopamine (mediated by suprathreshold salient footshock stimulation). In contrast, an optimal level of $D_4R$ stimulation (dotted line) would enable the encoding of emotional associative fear memories. Optimal stimulation resulted from an effective intra-mPFC dose of the $D_4R$ agonist administered before subthreshold mild footshock conditioning, or the actions of endogenous dopamine mediated by strong suprathreshold footshock stimulus. Figure adapted from Zahrt et al. (1997).

encoding of a conditioned fear response remains to be elucidated. Optimal $D_4R$ activation together with synchronized input from BLA afferents is necessary for the encoding of such responses, indicating that $D_4R$ activity modulates information processing in this pathway. The recent work of Lauzon et al. (2009) has enhanced our current understanding of the dopamine mechanisms mediating associative memory formation within the mPFC and provides important directions for future research. Whereas the cellular mechanisms remain to be determined, appropriate dopamine stimulation of mPFC $D_4Rs$ appears to be critical for the encoding of emotionally salient cues. Thus, either deficient or excessive $D_4R$ stimulation may produce marked mPFC dysfunction, with important implications for dopamine mechanisms involved in neuropsychiatric illness. As the cellular mechanisms governing this inverted U-shaped response become established, pharmacological targets could be devised to reestablish the physiologic balance between salient and nonsalient emotional information processing disrupted in neuropsychiatric disorders such as schizophrenia, depression, and drug addiction.

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