Using DREADDDs to isolate internal clocks

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Although much is known about the psychophysics of timing and time perception, the ability to selectively modulate discrete neuronal populations subserving interval timing could advance the field in unprecedented ways (see Buhusi and Meck, 2005; Allman and Meck, 2011; Cheng et al., 2011). Current approaches used to delineate the neuronal correlates of timing are often restricted to pharmacological (Meck, 1996; Coull et al., 2011) or ablative approaches, both physical (Meck, 2006a,b; Wiener et al., 2008) and genetic (Sysoeva et al., 2010; Agostino et al., 2011; Meck et al., 2011; Wiener et al., 2011). Most, if not all, of the ablative approaches are limited to loss of function-type hypothesis testing and frequently have unintended off-target effects. The pharmacologic approach has inherent confounds in the non-specificity of small molecule ligands. One way to overcome these confounds is to use a system in which a designer receptor exclusively activated by designer drug (DREADD) is expressed in a cell-type specific manner via transgenic expression. This chemicogenetic approach is further described by the acronym of the first tool of its type: receptor activated solely by synthetic ligand (RASSL). In short, a receptor is engineered such that it is no longer activated by any endogenous ligands and instead activated by an otherwise inert exogenous ligand. The various RASSLs and DREADDDs have been reviewed elsewhere (Fei et al., 2008). In short, there are three DREADD available with proven functionality in neuronal environments – hM3Dq (Gq-coupled, promotes neuronal excitability), hM4Di (Gi-coupled, promotes neuronal inhibition), and the rM3Ds (Gs-coupled, promotes cAMP production). Here, we discuss this technology’s potential in the field of interval timing.

Cell-type specificity of signaling has been a hurdle for delineating the neuroanatomical substrates of timing (Cheng et al., 2011). Using the chemicogenetic approach, it is possible to express a receptor in a select population of neurons and then turn that receptor on via peripheral administration of clozapine-N-oxide (CNO). The cell-type specificity is dependent upon the transgenic approach used, though the current genetic toolkit appears to be more than adequate for the study of interval timing. To date, there has been success expressing DREADDDs using virally mediated approaches (Ferguson et al., 2011; Krashes et al., 2011; Sasaki et al., 2011), single-transgenic approaches (Guettier et al., 2009), and intersectional transgenic approaches (Alexander et al., 2009; Ray et al., 2011). With these approaches, it should be possible to place a DREADD into a subpopulation of neurons and then determine how this population modulates interval timing. Furthermore, the identical signaling-type initiated via the DREADDDs in various subpopulations would allow us to piece together the circuitry by expressing the DREADD in multiple subpopulations either simultaneously or separately (Figure 1).

In addition to the cell-type specificity possible with DREADDDs, this approach also affords us the ability to silently and independently modulate neuronal signaling. An inherent confound in the pharmacologic approach to neuronal modulation is the intrinsic interference with the endogenous tone of the system being investigated. By definition, any agonist interacting with a receptor competes with the endogenous tone, leaving the theoretical temporal encoding in place (Figure 2C). This provides us the unique opportunity to reductively piece together each component of the neuronal substrates of timing.

Finally, the usability of the chemicogenetic approach is another salient aspect when considering its application in the field of interval timing. Indeed, other approaches for cell-type specific control of neuronal signaling exist, most notably that of optogenetics (Yizhar et al., 2011). The photoexcitative approach has many features that lend well to interval timing research — namely, the exclusive control of neuronal firing in the subsecond range. Both optogenetics and chemicogenetics achieve cell-type specific control of signaling through transgenic expression of a non-native receptor, so both approaches leave the endogenous tone intact. The chemicogenetic approach, in its simplest form (a single-transgenic mouse), requires a simple peritoneal injection of CNO to modulate cell-type specific neuronal signaling. This simplicity is an experimental aspect that cannot be ignored considering the exclusive use of behavior paradigms in the study of interval timing.
In conclusion, it can be seen that by combining DREADDs and transgenic technology in the chemogenetic approach it will be possible to further delineate the neuronal substrates of interval timing. The cell-type specific control in combination with the independent modulation of neuronal signaling afford a level of precision that is almost requisite in the investigation of such processes.
an intertwined neuronal process as that of interval timing. We propose an initial experiment here: investigating the functional role of the MSNs in temporal integration (see Matell and Meck, 2004; Oprisan and Buhusi, 2011). It can be seen that this technology is well suited for use in this field, and will hopefully advance our understanding of the neural basis of timing and time perception (Gibbon et al., 1997; Gu et al., 2011).

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