LETTER

Report of interval timing or action?

Xu et al. (1) argue that the medial prefrontal cortex (mPFC) is involved in interval timing. However, we believe that their data can be explained by the hypothesis that the mPFC simply responds to port exit: the action indicating the expiry of timed intervals. To illustrate our argument, imagine the following addition to the experiment: a flash of light is presented to animals when they exit the waiting port. Say that neurons in the primary visual cortex (V1) respond to the light, as expected. If one were to plot a peri-event time histogram (PETH) of these responses aligned to the sound onset (as in figure 3 of ref. 1), neurons in V1 would show an increase in firing around the exit, when the light is delivered. Importantly, the time waited by the animals until they exit and receive the light will exhibit some variability. Because timing behavior follows the scalar timing property (2, 3), this variability will be proportionally greater for longer intervals. When a PETH is averaged across trials, the variability in the sound-to-light interval will influence the PETH in a scaled fashion: a more variable interval corresponds to a gentler slope. The form of the neural response in the sound-aligned PETH could therefore merely reflect the distribution of times to an acute visual response following the sound, not a ramping of activity facilitating interval timing. This hypothetical result is similar to figure 3 in ref. 1, yet would one conclude that neurons in V1 represent the interval waited and not the visual stimulus? Figure 2 in ref. 1 shows that the analyzed neurons clearly respond to the port exit. Hence, the conclusion that mPFC neurons represent the interval waited by animals has a major flaw: neural responses may merely convey the state of being in the port, with the scalability of their responses resulting from averaging scaled variability in exit times. To demonstrate a neural instantiation of interval timing in this experiment, it has to be shown that on trials in which the exit isn't timed, neurons do not respond to the port exit. This may be done in the following two ways: (*i*) requiring animals to exit the port immediately after a different cue; and (*ii*) presenting catch trials without a test sound so that exits cannot be timed from the sound. Without these controls to rule out exit-related responses, the most parsimonious explanation is that neurons in the mPFC merely respond to the port exit.

Importantly, Xu et al.'s (1) paper offers a manipulation of the brain region hypothesized to inform timed behavior by cooling mPFC and evidencing a right-shift in the distribution of wait-times. However, because the PFC is thought to "orchestrate thought and action in accordance with internal goals" (4), this too is indeterminate in establishing mPFC's role in timing because it may (*i*) reduce the ability to sense the sound onset or presence in the port, (*ii*) slow the decision to exit, or similarly, (*iii*) slow the decision to initiate timing, while in all cases leaving interval timing—itself—intact. In the absence of such controls, it is impossible to ascertain whether the results of the cooling experiment are truly a result of impaired interval timing.

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