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Temporal processing in the striatum: interplay between midbrain dopamine neurons and striatal cholinergic interneurons

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List of abbreviations:

DA: dopamine

ChI: cholinergic interneuron

TAN: tonically active neuron

MSN: medium spiny neuron

SBF: striatal beat frequency

Abstract

There has been considerable progress in recent years towards understanding the neuronal mechanisms mediating time perception. Notably, the striatum and its dopamine (DA) input from the ventral midbrain are considered to be central for timing on the scale of hundreds of milliseconds and seconds. The cholinergic interneurons (ChIs) of the striatum provide an extensive local innervation which closely interacts with striatal DA afferents. Both neuronal systems have been shown to influence synaptic plasticity to shape the transfer of information through the striatum. Given their cooperative role in regulating striatal output pathways, DA and cholinergic inputs may have distinct but complementary roles in timing processes. Electrophysiological recordings from behaving animals have provided evidence that responses of midbrain DA neurons and striatal tonically active neurons (TANs), presumed ChIs, to motivationally relevant events are sensitive to the predicted time of these events. Namely, changes in neuronal activity are reduced or absent at times when events are more expected, indicating that temporal aspects of prediction play an important role in the responsiveness of these two neuronal systems. Recently, new findings have further suggested that DA neurons and cholinergic TANs are both involved in the ability to keep track of elapsed time. These two systems appear to work in parallel in initiating the timing process at the beginning of an interval to be timed. It therefore appears that DA and ChI signaling could participate in striatal processing that is crucial for the control of timing behavior.

Introduction

Information about timing is encoded in a distributed network of brain regions including the basal ganglia, cerebellum and multiple areas of the frontal and parietal cortices (Buhusi and Meck, 2005; Coull *et al.*, 2011; Merchant *et al.*, 2013). Numerous lines of evidence, from human neuroimaging studies to inactivation experiments in animals, suggest that the striatum, the main recipient of afferents to the basal ganglia, plays a central role in interval timing, defined as the perception of durations in the range of hundreds of milliseconds to several seconds. The main population of neurons in the striatum, the GABAergic projection neurons, also called medium spiny neurons (MSNs), has been at the center of research on how striatal circuits could convey information about the passage of time. Previous electrophysiological studies reporting neuronal correlates of timing in the striatum have focused on MSNs in rodents (Matell *et al.*, 2003; Mello *et al.*, 2015; Gouvêa *et al.*, 2015; Bakhurin *et al.*, 2017) and monkeys (Jin *et al.*, 2009; Chiba *et al.*, 2015; Wang *et al.*, 2018), whereas the role of local interneuron circuits that exert influence on striatal function has not been considered. In particular, cholinergic interneurons (ChIs) have long been known to be important, along with DA input, for various forms of corticostriatal synaptic plasticity (Di Filippo *et al.*, 2009) and they play a powerful role in modulating DA release in the striatum (Cragg, 2006; Threlfell *et al.*, 2012; Cachope & Cheer, 2014). Although they represent only ~3% of the total neuronal population in the striatum, ChIs give rise to extensive axonal arbors that exert an important regulation on striatal output in cooperation with DA (Calabresi *et al.*, 2000; Pisani *et al.*, 2007). Even though theoretical models of interval timing have tried to specify a role for the striatum and its DA input in the control of timing behavior, they do not outline how ChIs contribute to the neuronal mechanisms that underlie timing functions. The present review summarizes the relevant data linking time processing with DA and cholinergic transmissions in the striatum. We particularly

focus on recent neuron recording studies from midbrain DA neurons and striatal ChIs that seek to clarify how these two neuronal systems contribute to timing processes.

Involvement of striatal dopamine transmission in temporal processing

Numerous studies have suggested that timing mechanisms depend on the integrity of midbrain DA neurons, particularly those projecting from the substantia nigra *pars compacta* to the dorsal striatum (Meck, 1983; 1996). Abnormal temporal processing has been reported in pathological conditions associated with DA dysfunction, such as Parkinson's disease (Pastor *et al.*, 1992; Parker *et al.*, 2013; Merchant *et al.*, 2008; Mioni *et al.*, 2018), schizophrenia (Rammsayer, 1990; Volz *et al.*, 2001; Davalos *et al.*, 2003; Hwang *et al.*, 2010), and attention deficit disorder (Yang *et al.*, 2007; Hwang *et al.*, 2010; Smith *et al.*, 2013). Pharmacological studies in both animals and humans and lesion studies in animals have extensively documented the link between DA and temporal processing (Meck, 1996). For example, impaired timing variability has been recently reported after the systemic injection of the dopamine D2 receptor agonist quinpirole in monkeys trained to produce rhythmic movements (Yc *et al.*, 2019). In addition, there is evidence for impaired timing behavior after specific inactivation of substantia nigra DA neurons, using optogenetic and chemogenetic approaches in mice trained to discriminate the duration of time intervals (Soares *et al.*, 2016). Also, a neuroimaging study in human subjects performing a timing task has shown that a pharmacologically-induced reduction of DA levels can decrease the time-dependent activation within the striatum, in parallel with disturbances in timing ability (Coull *et al.*, 2012).

The most widely accepted explanation about how DA activity intervenes in temporal processing is that striatal DA levels modulate the speed of a putative internal clock supposedly designed to produce a representation of elapsed time (Merchant *et al.*, 2013; Paton & Buonomano, 2018). According to this view, decreased striatal DA transmission, in response to

pathological or experimental situations, is associated with a reduction in clock speed (i.e., a slowing of temporal processing) leading to an overestimation of time, and the reverse occurs in case of increased DA transmission associated with underestimation (Meck, 1983; 1996). Although this internal clock model explains a number of behavioral results obtained in a variety of timing tasks, in both animals and humans, the underlying neurobiological mechanisms were not specifically addressed. A more elaborate view has been proposed to connect theoretical accounts of temporal processing with possible neuronal substrates. The so-called *striatal beat frequency* (SBF) model of Matell and Meck (Matell & Meck, 2000; Matell *et al.*, 2004) highlights the relationship between corticostriatal circuits and midbrain DA neurons. The model assumes that detection of coincident cortical activity by MSNs may encode the representation of elapsed time. The start signal to begin timing is considered to be marked by a phasic activation of DA neurons, acquired through learning, that synchronizes the oscillations of cortical neurons at the onset of the interval to be timed. The SBF model mainly focuses on the influence of cortical neuronal oscillators on MSNs located in the dorsal striatum under the influence of DA signals. However, while the idea is interesting, there is little physiological evidence supporting such a relation between MSN activity and cortical oscillatory activity and the model thus remains largely speculative. In sum, the above models of timing propose a central role for the DA transmission, with an influence that arises at different stages of time processing according to the model. The most popular theory (clock model) considers that the level of DA modulates the speed of an internal time keeping mechanism, whereas another theoretical perspective (SBF model) assumes that the DA modulation serves primarily as a start signal to keep track of elapsed time. Despite the close interplay and dependence between the two neuronal systems in the striatum, it is noteworthy that both theoretical models focus exclusively on the implication of DA input and do not account for a specific role of striatal cholinergic transmission in time processing.

A more recent theoretical view considers that striatal encoding of elapsed time relies on dynamic changes in the population of MSNs, a model referred to as « population clocks » (Buonomano & Karmarkar, 2002). Although there is a growing body of experimental evidence in favor of such a population coding (Gouvêa *et al.*, 2015; Mello *et al.*, 2015; Bakhurin *et al.*, 2017; Wang *et al.*, 2018), the link between DA and cholinergic modulations and neuronal dynamics in striatal output circuits has yet to be clarified.

Involvement of striatal cholinergic transmission in temporal processing

Compared to DA, far less is known about the role that cholinergic transmission exerts on the encoding of time. Pharmacological manipulations by systemic injections of cholinergic drugs in rodents have established a link between cholinergic activity and timing behavior (Meck, 1983; 1996; 2002; Meck & Church, 1987). Within the framework of the internal clock model and based on the dynamics of the observed effects on timing behavior, it has been suggested that cholinergic manipulations impair timing by disturbing storage of temporal information into memory. These data led to the proposal that DA neurons of the substantia nigra projecting to the dorsal striatum are involved in temporal processing speed, whereas cholinergic neurons of the basal forebrain projecting to the frontal cortex are thought to be important for the temporal memory representation (Meck, 1996; Meck & Church, 1987; Coull *et al.*, 2011; Oprisan & Buhusi, 2011). However, systemic pharmacological injections are unspecific and do not allow to determine the precise contribution of the intrinsic cholinergic innervation of the striatum, thus requiring a more selective approach by using local microinjections. Some studies have used local application of cholinergic receptor antagonists into the striatum (Ztaou *et al.*, 2016) or targeted inactivation of ChIs over different regions of the striatum (Okada *et al.*, 2014; Aoki *et al.*, 2015; 2018), but they did not address the issue of the role of ChIs in timing processes; rather there is a current, growing interest in the role of these interneurons in the control of

flexible switching of behaviors (Brown *et al.*, 2010; Prado *et al.*, 2017; Bell *et al.*, 2019) and monitoring of current environmental state during behavior (Apicella, 2007; Bradfield *et al.*, 2013; Stalnaker *et al.*, 2016), leaving aside the question of their role in time processing. Interestingly, disturbances in timing behavior have been reported in patients with Tourette syndrome (Vicario *et al.*, 2010), a neuropsychiatric disorder in which the density of specific types of striatal interneurons, including ChIs, has been shown to be decreased (Kataoka *et al.*, 2010).

Single neuron recordings in behaving animals

More direct evidence in support for the contribution of midbrain DA neurons and striatal ChIs to the encoding of time has come from electrophysiological studies in which animals were exposed to variation in the timing of rewarding events. Studies in monkeys have demonstrated that receipt of a reward at a fully expected time elicits little or no change in the activity of DA neurons, whereas a brief increase in firing rate emerges when the timing of reward becomes less predictable (Hollerman & Schultz, 1998; Fiorillo *et al.*, 2008; Kobayashi & Schultz, 2008). This is illustrated in Fig.1A in which reward delivered earlier or later than its usual time led to a strong DA response. Other studies have reported that DA responses are reduced as the occurrence of a behaviorally relevant stimulus becomes increasingly predicted over time, a phenomenon referred to as the hazard rate (Fiorillo *et al.*, 2008; Pasquereau & Turner, 2015). As a whole, these data indicate that the strength of DA responses is shaped by the degree of temporal predictability of rewarding events.

Most of the ideas about the role of the intrinsic striatal cholinergic innervation come from recordings of tonically active neurons (TANs) that are presumed to be ChIs. TANs can be distinguished from other striatal neurons based on distinguishable electrophysiological features and changes in activity in response to rewarding events (Apicella, 2002). Whether there are

other striatal interneurons than ChIs among the group identified as TANs remains a possibility given limitations of current electrophysiological recording techniques in behaving monkeys to probe the identity of sampled neurons (Apicella, 2017). As for DA neurons, TANs participate in signaling stimuli predictive of reward and the reward itself. An important property of TAN responses is their dependency on the temporal predictability of rewarding events. As reported in our earlier studies, TAN responses to reward, consisting mainly of brief decreases in firing rate (the so-called "pause"), were reduced or even abolished when the timing of reward can be predicted in Pavlovian and instrumental tasks (Apicella *et al.*, 1997; 2006; Ravel *et al.*, 2001). As can be seen in the example neuron shown in Fig. 1B, the TAN response to reward increased with time intervals shorter or longer than the habitual one, in a manner quite similar to what has been described for DA neurons. Similarly, in an arm reaching task, the time delay between an instruction cue and the subsequent movement-triggering stimulus is a major determinant of TAN responses, with lengthening the delay having an increasing effect on the response to the trigger and a decreasing effect on the response to the cue (Sardo *et al.*, 2000). It is therefore evident from this brief overview of their response properties that DA neurons and ChIs are both sensitive to temporal aspects of stimulus prediction. However, we lack information about their precise contribution to the striatal mechanisms mediating temporal information processing.

The role of midbrain dopamine neurons and striatal cholinergic TANs in the processing of temporal information

In the research reviewed above, DA and ChI systems were studied in the context of tasks that required animals to use temporal regularities in sequential events to predict the onset of a reward-predicting stimulus or the reward itself. In such conditions, the representation of elapsed time could be assumed to be "implicit", as it relies upon predictive temporal relations between events acquired through extensive practice. There are very few studies that have evaluated the

contribution of DA neurons and striatal ChIs to timing under conditions that require an overt estimate of elapsing time to guide behavior, thus focusing on "explicit" timing mechanisms rather than "implicit" ones (Coull & Nobre, 2008; Coull *et al.*, 2011).

Only one study has investigated DA activity in a condition in which animals were required to produce an overt estimate of elapsed time (Soares *et al.*, 2016). The researchers trained mice to categorize the durations of intertone intervals as shorter or longer than a pre-learned duration (Fig.2A). During this temporal discrimination task, DA activity in the substantia nigra *pars compacta* was measured using fiber photometry-based calcium recording. As can be seen in Fig.2B, DA activity increased after the first and the second tone defining the time interval and this increase was stronger when the interval was judged as shorter, reflecting an underestimation of the duration of the interval. Conversely, a weaker DA increase was observed when the interval was judged as longer (i.e., overestimation). This suggests that higher or lower level of DA activity was closely related to the animals' estimates of the duration of time intervals (Fig.2C). In the same study, the authors demonstrated that the observed changes in DA activity are functionally relevant because optogenetic manipulations of nigral DA signaling affect timing performance. This is the first study to demonstrate a link between DA activity with the animal's ability to react in a temporally controlled manner.

As regards striatal ChIs, a number of recording studies have reported changes in TAN activity that are dependent on temporal prediction, but in none of them were animals required to use temporal information to guide behavior. Recently, we have recorded from single TANs in the striatum of two rhesus monkeys trained to perform a timing task in which they had to initiate self-timed reaching movements after a specified time interval has passed (Martel & Apicella, unpublished results). Animals were required to produce an appropriate time interval that was close to a pre-learned minimum waiting period before initiating an action (Fig.3A *left part*), a performance that relies on internally scaled estimates of duration. We studied 200 TANs

in this duration estimation task using recording methods described in detail previously (e.g., Apicella et al., 2011). About one third of the recorded TANs (73 of 200 neurons, 37%) displayed significant changes in activity in response to the cue that indicates the beginning of the time interval, essentially consisting of a decrease in firing rate. This pause response was similar in magnitude regardless of whether the cue was associated with an ensuing short or long interval (Fig.3B). Also, we found no evidence that the TAN response to the cue might reflect the monkey's accuracy of estimating interval duration (Fig.3C). Although studies in rodents and monkeys are difficult to compare because of differences in task design, the lack of sensitivity of TANs to timing accuracy appears to contrast with the level of DA activity which is predictive of performance during time-guided behavior (Soares *et al.*, 2016).

In the same monkeys, we also tested the effects of interval durations when animals were passively exposed to a visual stimulus followed by the delivery of reward in a Pavlovian conditioning task (Fig. 3A *right part*). This design allowed us to look specifically at the influence of a stimulus providing advance information about the timing of reward in a condition that excludes possible confounding factors that were linked to target reaching. A total of 90 TANs were recorded in the Pavlovian protocol and 38 of them (42%) responded to the stimulus that serves as a timing cue. At the population level, the TAN pause response (Fig.3D) was essentially similar to that observed in the duration estimation task. However, by focusing on a subset of 31 TANs that were tested in both testing conditions, we found that subsets of TANs responded to the timing cue selectively in one condition, either duration estimation task or Pavlovian protocol (Fig.3E). That a substantial fraction of TANs responded only in one testing condition supports the hypothesis that responses do not strictly follow timing cues themselves, but rather do so according to the contextual demands of the task. Such a context dependency in TAN activity is a recurring topic of discussion in the literature on the role of the ChIs (Apicella, 2007; Bradfield *et al.*, 2013; Stalnaker *et al.*, 2016). Our results are consistent with the idea that

the cholinergic TAN system might support recognition of previously learned temporal contexts. However, although changes in TAN activity have been hypothesized to be under the control of context, the mechanisms through which these interneurons use context detection to regulate striatal output and potentially influence timing behavior are far from being understood. In particular, there are difficulties in most neurophysiological studies to isolate the encoding and processing of temporal information from the detection of the context. To better test whether TANs preferentially encode the context or time, it would be conceivable to use a task design in which precise timing of actions or rewarding events can occur in different contexts. For example, one stimulus (temporal cue) instructs the animal about the duration of the delay prior to movement, while another stimulus (contextual cue) indicates that the animal must make or withhold movement to obtain a reward (a condition that could be referred to as a “time-dependent Go-NoGo task”). Using trial blocks in which the delay time and reward contingency are independently varied would make it possible to compare the effects of each task variable on TAN activity.

The contribution of dopamine-acetylcholine interactions to temporal processing in the striatum

As mentioned in this review, there is experimental evidence from pharmacological and lesion studies indicating that manipulations of DA and cholinergic transmissions cause disturbances in timing behavior. It is mainly on the basis of these results that it has been proposed that DA mediates the speed of an internal clock, whereas acetylcholine would be rather involved in encoding temporal memory (Meck, 1996; Buhusi & Meck, 2005) or in detecting a learned context (Apicella, 2007; Bradfield *et al.*, 2013; Stalnaker *et al.*, 2016). On the other hand, a more direct assessment of the activity of DA neurons (Soares *et al.*, 2016) and ChIs during

timing tasks suggests that these two neuronal systems appear to work in parallel in initiating the timing process at the beginning of an interval to be timed. In this regard, midbrain DA neurons and striatal ChIs may provide a signal potentially suitable for time processing in the striatum. However, unraveling their differential sensitivity across studies is difficult because of variations of experimental designs, notably the type of behavioral procedure used to investigate interval timing in rodents and primates. In addition, it should be underscored that both DA neurons and ChIs participate in the encoding of reward prediction error and they are both central to reward-guided learning. The intertwining of reward prediction and temporal processing is the source of difficulties in distinguishing neuronal signals related to expectations about future rewards from those related to timing processes (Paton & Buonomano, 2018). This needs to be taken into account for the interpretation of the results obtained in tasks used to assess the ability of animals to time interval durations.

As emphasized above, DA and cholinergic transmissions have strong reciprocal actions on each other, but the functional outcome of these interactions and their impact on striatal output need to be further explored. There is evidence indicating that phasic changes in activity of DA neurons and ChIs tend to occur simultaneously and it has been put forward that ChI signaling would serve as a temporal frame defining the time over which the information carried by the DA signal is processed in striatal target regions (Morris *et al.*, 2004; Cragg, 2006). Although this hypothesis has attracted much attention, further work needs to be done to understand the ways in which DA neurons and ChIs carrying temporal information act on striatal output pathways that are responsible for sending motor commands. A crucial issue for future research is to better understand the precise engagement of each system in timing behavior, for example, by using genetic tools that are capable of targeting the local cholinergic innervation of the striatum or DA midbrain projections to the striatum to assess causal effects of selectively disrupting one or the other neuronal system.

Although DA and cholinergic transmissions have been shown to elicit changes in synaptic strength taking place within the striatal circuitry, virtually nothing is known about how these cellular mechanisms may underlie, at least in part, the processing of temporal information. One can only speculate on the role of striatal synaptic plasticity as a potential mechanism for the acquisition and maintenance of temporal memories and controlling timing behavior. Notably, in the framework of the SBF model, it has been proposed that synaptic connections are strengthened or weakened in order to produce a record in memory of learned durations (Matell & Meck, 2004). Investigating how mechanisms of synaptic plasticity may impact performance during timing tasks are key open areas of research within the time perception field.

In conclusion, the striatum continues to elicit a great deal of interest from neuroscientists as a brain region essential for the processing of temporal information. Here, we have briefly reviewed recent advances in deciphering how DA neurons and striatal ChIs may participate in the encoding of time. Future studies could aim to precisely define the key aspects of timing tasks that selectively influence the activity of these two neuronal systems. In particular, one major challenge is to disentangle neuronal activities related to timing processes from motivation, attention, and action selection, since it is well known that DA and ChI systems are involved in reward-guided learning. It is noteworthy that encoding time over ranges of seconds to minutes is required for learning to predict rewards and to guide behavior toward rewarding outcomes. In this regard, a more detailed analysis of interactions between timing and reward processing is a promising direction for future work and computational models for integrating these two aspects will also provide substantial help in clarifying the respective roles of midbrain DA neurons and striatal ChIs (Daw *et al.*, 2006; Petter *et al.*, 2018).

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Conflict of interest

The authors declare no competing interests.

Authors contributions

A.C.M. and P.A. wrote the manuscript.

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Figure legends

Figure 1. Modulation of dopamine neuron and cholinergic interneuron activity when reward occurs at a different time as predicted. Monkeys were trained on an instrumental task (**A**) or a Pavlovian conditioning task (**B**) in which a lever contact or a visual stimulus is followed, after a constant time interval (1s), by the delivery of a liquid reward. During these experiments, the interval was occasionally shortened or lengthened across successive trial blocks. Each line of dots indicates the neuronal activity during a single trial aligned on the onset of the lever contact or visual stimulus. The sequence of trials is shown chronologically from top to bottom in each block. Both neurons did not change their activity when reward was delivered at the end of the accustomed 1-s delay, whereas they displayed a brief increase (**A**) or decrease (**B**) in activity when reward was delivered earlier or later than the usual time, suggesting a sensitivity to temporal aspects of reward prediction. The DA neuron also showed a decrease in activity at the habitual time of the reward when it was delayed beyond the expected time, reflecting the known role of these neurons in reward prediction error signaling (i.e., the difference between expected and received reward). The cholinergic TAN also responded to the visual stimulus predictive of reward, and this response was unchanged across the different trial blocks. (**A**) modified from Hollerman and Schultz 1998; (**B**) modified from Apicella 2017.

Figure 2. Modulation of dopamine neuron activity during a temporal discrimination task. **A.** Task event sequence. Mice were trained to keep track of the time elapsed between two auditory stimuli in order to make decisions as to whether the intertone interval was shorter or longer than a pre-learned 1.5-s interval duration. Animals' judgements about the duration of time intervals (i.e., shorter or longer) were expressed as movements toward one or the other side. The gray-shaded area indicates the interval reference. **B.** Photometric recordings of DA neuron activity during task performance. Trials were divided into three groups (High, Med, and Low) according

to the strength of the DA response to the second tone for a given interval (1.74 second). **C.** Psychometric curves corresponding to trials from each group. The magnitude of the DA response increased when mice judged the duration of time intervals as being shorter than the memorized standard (i.e., underestimation). The inset shows the difference in the probability of making a long choice between Med and Low or High (red or blue) DA responses. Adapted, with permission, from Soares et al. 2016.

Figure 3. Modulation of cholinergic TAN activity during two timing task. **A.** Sequence of task events. *Left*, In the duration estimation task, the monkey kept the hand resting on a bar and a visual stimulus on the left or the right side marked the beginning of a particular time interval (short or long, in the seconds range) with the cue location indicating interval duration. The monkey had to wait until the time interval has elapsed before reaching the target on the side of the stimulus. *Right*, In the Pavlovian protocol, the presentation of a visual stimulus was automatically followed by reward at the end of the same two time intervals as in the duration estimation task. The areas shaded gray represent the minimum waiting time before self-initiated movements (*left*) and the duration of the stimulus-reward interval (*right*). **B.** Population-averaged responses of TANs to the timing cue in the duration estimation task, separately for short and long intervals. The vertical dotted line indicates the cue onset. n, number of neurons. **C.** Influence of accuracy of duration estimation on the magnitude of the TAN response. Trials were divided into different groups according to movement onset time and magnitudes of change were calculated to include only the trials within a specific range, separately for short and long intervals. Movements starting before the movement onset threshold (-500/0 range) were not rewarded. The magnitude of the pause response did not differ among different trial ranges in the population of recorded TANs. **D.** Population-averaged responses of TANs to the timing cue in the Pavlovian protocol, separately for short and long intervals. The vertical dotted line

indicates the onset of the reward-predicting stimulus. n, number of neurons. **E.** Selectivity of TAN responses to the timing cue for a particular condition. The percentages are calculated from the total number of TANs recorded in both the duration estimation task and the Pavlovian conditioning tasks. n, number of neurons. DET, duration estimation task; PCT, Pavlovian conditioning task. Martel and Apicella, unpublished results.

Figure 1

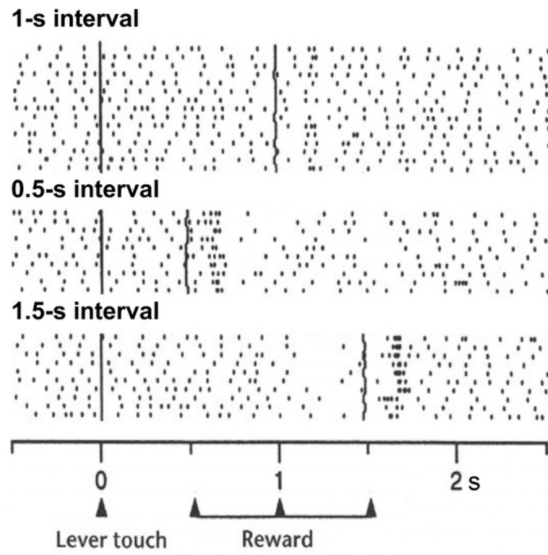
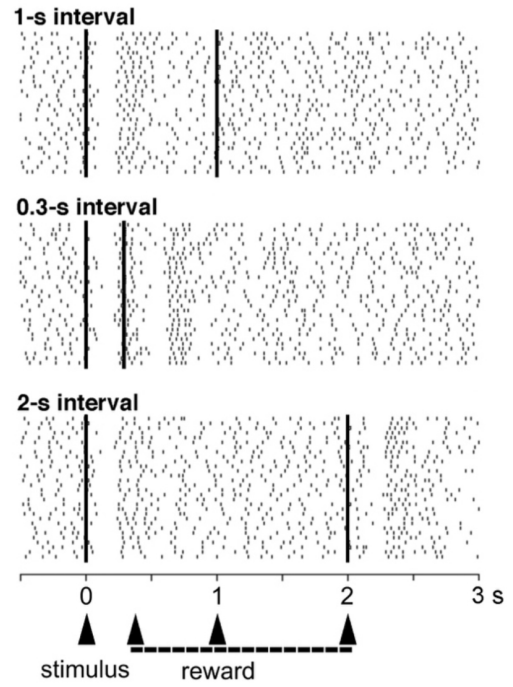
A midbrain dopamine neuron**B** striatal cholinergic interneuron

Figure 2

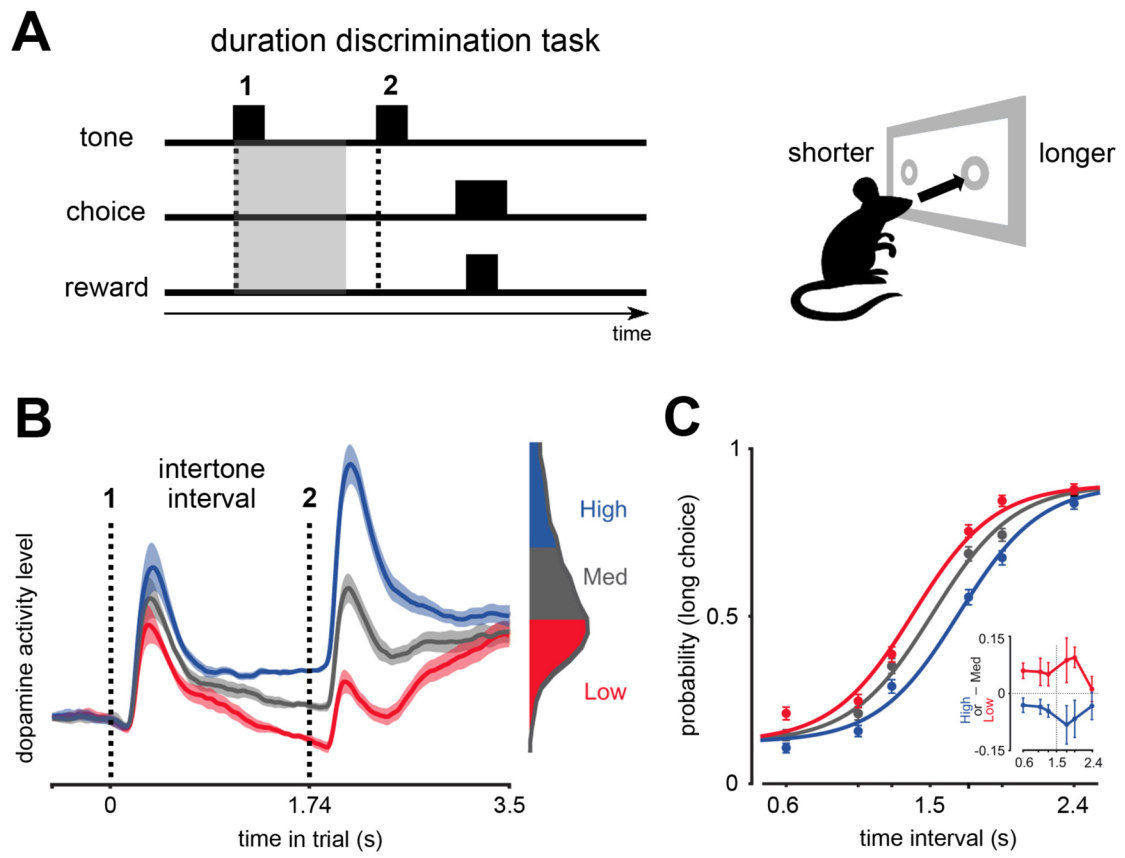


Figure 3

