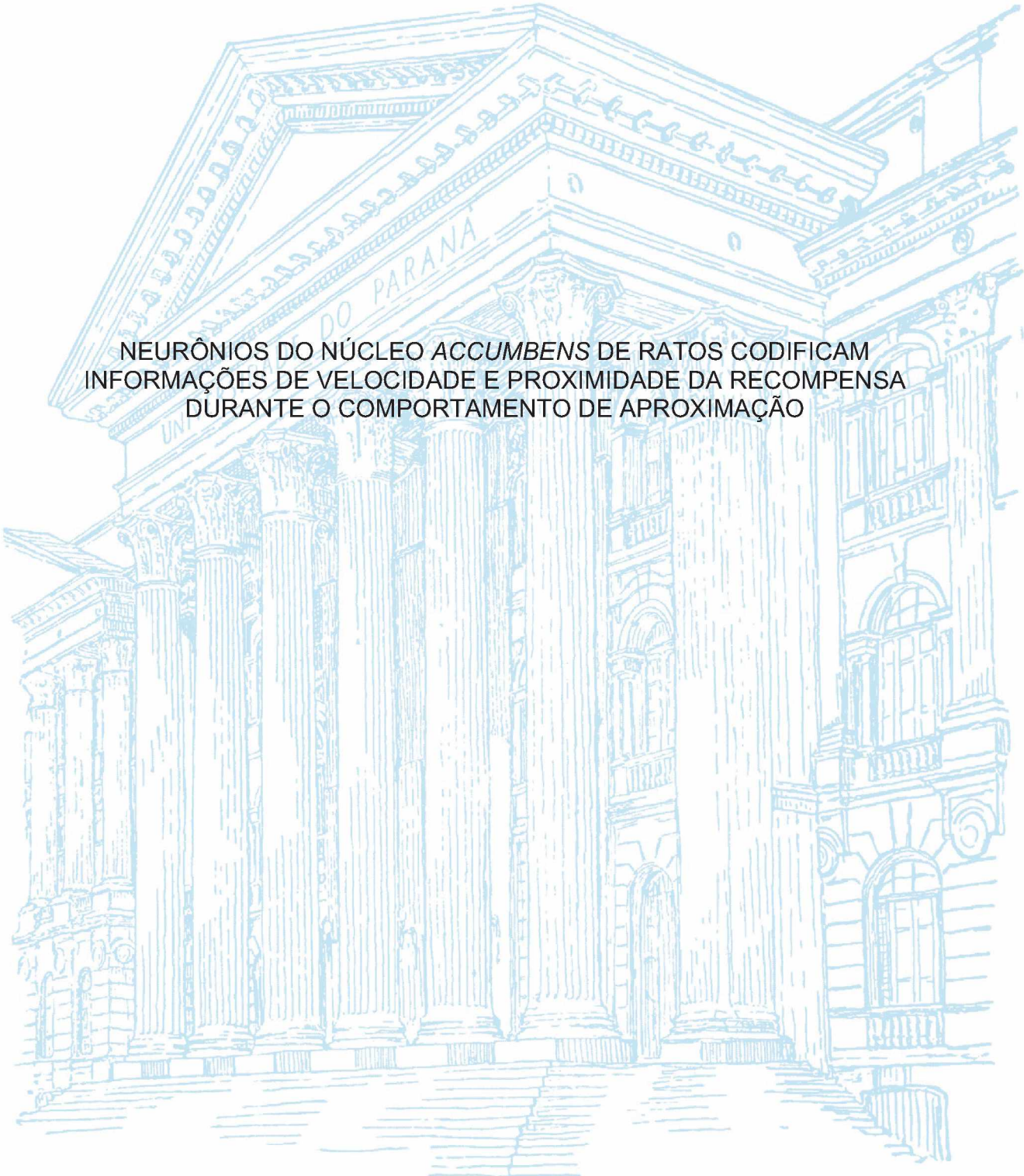
UNIVERSIDADE FEDERAL DO PARANÁ

ADAM HIDEO SUGI



NEURÔNIOS DO NÚCLEO *ACCUMBENS* DE RATOS CODIFICAM INFORMAÇÕES DE VELOCIDADE E PROXIMIDADE DA RECOMPENSA DURANTE O COMPORTAMENTO DE APROXIMAÇÃO

CURITIBA

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Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Ciências - Bioquímica, no Curso de Pós-Graduação em Ciências - Bioquímica,Setor de Ciências Biológicas, da Universidade Federaldo Paraná.
Orientador: Prof. Dr. Cláudio da Cunha

CURITIBA

2019

Universidade Federal do Paraná. Sistema de Bibliotecas.
Biblioteca de Ciências Biológicas.
(Giana Mara Seniski Silva – CRB/9 1406)

Sugi, Adam Hideo
Neurônios do núcleo *accumbens* de ratos codificam informações de velocidade e proximidade da recompensa durante o comportamento de aproximação. / Adam Hideo Sugi. – Curitiba, 2019.
61 p.: il.

Orientador: Cláudio da Cunha

Dissertação (mestrado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências - Bioquímica.

1. Núcleo accumbens 2. Recompensa 3. Comportamento apetitivo I. Título II. Cunha, Cláudio da, 1962- III. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências-Bioquímica.

CDD (22. ed.) 591.5

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS (BIOQUÍMICA) da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ADAM HIDEO SUGI** intitulada: **NEURÔNIOS DO NÚCLEO *ACCUMBENS* DE RATOS CODIFICAM INFORMAÇÕES DE VELOCIDADE E PROXIMIDADE DA RECOMPENSA DURANTE O COMPORTAMENTO DE APROXIMAÇÃO**, sob orientação do Prof. Dr. CLAUDIO DA CUNHA, que após após terem inquirido o aluno e realizado a avaliação do trabalho são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 28 de Agosto de 2019.

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DEDICATÓRIA

Dedico este trabalho aos meus pais como uma tentativa de agradecimento por todo o suporte e carinho.

AGRADECIMENTOS

Ao professor Doutor Claudio Da Cunha pela orientação, incentivo e suporte desde o inicio da iniciação científica.

Aos Professores Saleem Nicola e Romulo Fuentes pela essencial contribuição para este projeto.

Aos meus ex-colegas de trabalho David, Alex, Laura, Amanda, Dani e Julie que também me ensinaram muito e me guiaram durante boa parte a minha experiência na pesquisa.

Aos atuais colegas José, Gabriel, Mayra, William e João pelo café.

Aos amigos e amigas que fizeram destes anos de mestrado um período muito mais leve e feliz.

À Ana pela parceria, sinceridade e paciência, e que esteve e está sempre disponível para me escutar sobre as coisas boas e ruins.

Por fim, à minha família pelo enorme suporte que sempre será a base para qualquer conquista.

RESUMO

É proposto que o núcleo *accumbens* age como um interface entre emoções e ações motoras, sendo uma estrutura chave para o comportamento de procura de recompensas. É sugerido que a estrutura tem funções relacionadas à seleção de ações (e.g. para qual local se locomover) e/ou controle motor de respostas de aproximação (e.g. locomoção até alguma recompensa), entretanto, qual aspecto ou quais aspectos são codificados por estes neurônios ainda é pouco entendido. Neste trabalho, foi estudado, em uma tarefa de navegação espacial, o comportamento de aproximação envolvendo seleção de ação (escolher qual local ir) e as respostas neuronais do *accumbens*. Nós identificamos neurônios que codificam velocidade e proximidade do local recompensado, mas nenhuma evidência em relação a seleção de ação foi encontrada. Dentre os neurônios que codificavam essas informações (velocidade e proximidade), foi observado que a codificação destas variáveis não era homogênea, sugerindo que a informação não é codificada e/ou ela não chega igualmente para os neurônios do núcleo. Nossos dados favorecem a hipótese de que o *accumbens* possui um importante papel no controle motor do comportamento de aproximação, porém, nossas análises não revelaram nada relacionado à seleção de ações.

Palavras-chave: Nucleus Accumbens, navegação, comportamento de aproximação, recompensa, velocidade.

ABSTRACT

The *nucleus accumbens* is suggested to be an limbic-motor interface, and an key structure for reward seeking behavior. It is propose that the structure have a role in action selection (e.g. which place to go) and/or in the control of the motor response of the approach behavior (e.g. locomotion to the reward), however, which aspect or aspects are encoded by these neurons it is still poorly understood. Here we studied, in a spontaneous spatial navigation task, the approach behavior involving action selection (choosing places to go), and evaluated the responses of single neurons. We identified neurons in the *nucleus accumbens* which encode speed and proximity to the reward, but no action selection evidences were found. We also observed that the encoding of speed and reward proximity was not homogeneous among the recorded neurons, suggesting the information is not encoded or does not arrive equally in the *accumbens*. Our findings favors the hypothesis in which these neurons have a role in motor control of reward approach, however, our analysis did not show any action selection related activity.

Key-words: Nucleus Accumbens, navigation, approach behavior, reward, speed.

LISTA DE ABREVIATURAS E SIGLAS

Área tegmental ventral	- VTA
Cortex Pré-frontal	- CPF
Dopamina	- DA
Fast-scan cyclic voltammetry	- FSCV
Neurônios espinhosos médios	- MSN
Nucleo Accumbens	- NAc

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1. INTRODUÇÃO

Os núcleos da base consistem em um grupo de núcleos subcorticais que recebem informações de diversas áreas corticais e subcorticais e as envia para o córtex frontal e estruturas do cérebro relacionadas ao controle motor e respostas emocionais (DA CUNHA et al., 2012). O corpo estriado é a principal estrutura de entrada dos núcleos da base e sua atividade é importante para a seleção e execução de ações motoras (DA CUNHA et al., 2009, 2012; IKEMOTO et al, 2010; COX e WITTEN, 2019). Sinais motores de pacientes com a doença de Parkinson, tais como tremores, rigidez muscular e bradicinesia, também são atribuídos ao mau funcionamento dos circuitos que envolvem o estriado (ALBIN et al., 1989, FILLION et al., 1991, COSTA et al., 2006). Esses sinais motores da doença são atribuídos à morte de neurônios dopaminérgicos da região da substância negra pars compacta (CARLSSON, 1972), tendo por consequência uma diminuição da liberação de dopamina (DA) no estriado, causando assim os prejuízos motores (ALBIN et al., 1989). O estriado é composto por 95% de neurônios espinhosos médios (MSN, do inglês *médium spiny neurons*) GABAérgicos, os quais expressam receptores dopaminérgicos em suas membranas (KAWAGUCHI et al., 1995; SURMEIER et al., 2007), sendo assim, a liberação de DA apresenta um papel fundamental na modulação da atividade estriatal (SCHULTZ, 1997; BOSCHEN et al., 2011; WENDLER et al., 2014; IKEMOTO, 2010; DA CUNHA et al., 2015). Duas vias importantes relacionadas com a seleção de ação, fazem a comunicação entre o estriado e os núcleos de saída dos núcleos da base (globo pálido interno e globo pálido externo) são chamadas de via indireta e via direta e são moduladas pela dopamina (SURMERIER et al., 2007; DA CUNHA et al., 2015). A via indireta se inicia no estriado antes de chegar aos núcleos de saída passa pelo globo pálido externo e núcleo subtalâmico; a via direta também se inicia no estriado que se projeta de forma monosináptica para os núcleos de saída (DA CUNHA et al. 2009, 2012). Os núcleos de saída, por sua vez afetam a escolha de programas motores do córtex cerebral e de outros núcleos motores subcorticais. A comunição entre as vias de saída e os núcleos motores passa pelo tálamo ou

pelo globo pálido ventral. Os MSNs, constituem ambas as vias, entretanto, os neurônios cada via apresentam tipos de receptores dopaminérgicos distintos (KRAVITZ et al., 2010; NATSUBORI et al., 2017). MSNs da via direta expressam receptores tipo D1 que possuem uma menor afinidade pela dopamina, enquanto os neurônios da via indireta expressam receptores tipo D2 que possuem uma maior afinidade a dopamina (HU & WANG, 1988). Esta distinção na expressão dos receptores é vital para o funcionamento de ambas as vias, que por sua vez, modula a seleção e execução de uma ação motora (KRAVITZ et al., 2010).

Apesar da ampla quantidade de estudos demonstrando a importância do estriado para a seleção e execução de ações motoras, até mesmos trabalhos recentes utilizando a técnica de optogenética não são capazes de distinguir como os neurônios influenciam individualmente no comportamento motor. Alguns estudos recentes demonstraram a relação da atividade de neurônios com início e parada (Start/Stop) de ações motoras como locomoção, pressão de alavancas, e movimentação de membros superiores (PANIGRAHI et al., 2015; YTTRI e DUDMAN, 2016; SALES-CARBONELL et al., 2018). RUEDA-OROZCO e colaboradores (2015), também demonstrou a relação da atividade de neurônios do estriado dorsolateral com a velocidade e posição do animal durante a atividade locomotora apresentada nas corridas em uma esteira. Alguns desses estudos descreveram a atividade desses neurônios através de *tuning curves*, gráficos que mostram como a atividade neuronal que se "afina" para um determinado valor de uma variável (p. ex. velocidade) (Figura 1), porém, apesar de populações distintas de neurônios apresentarem *tuning curves* semelhantes (PANIGRAHI et al., 2015, SALES-CARBONELL et al., 2018), a relação entre esse padrão de disparo e o comportamento durante a execução da tarefa não está bem definida.

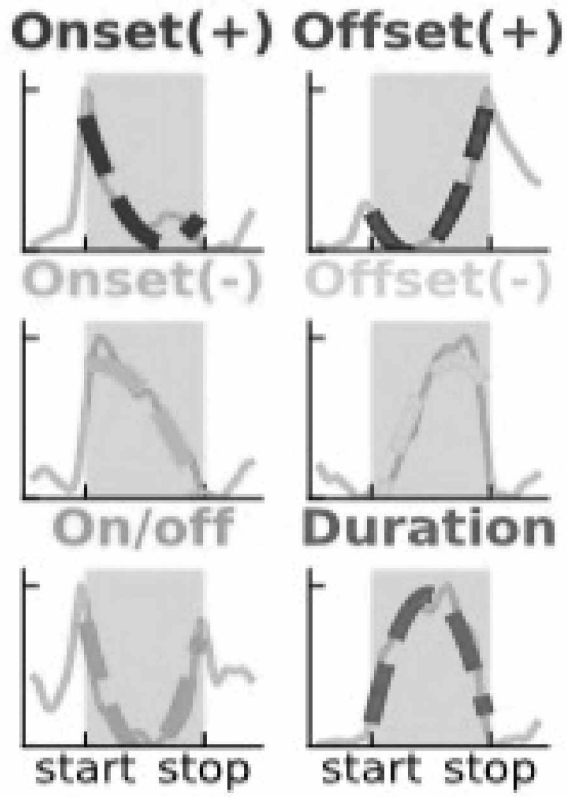


Figura 1. Exemplos de padrões de ativação de neurônios do estriado dorsolateral (SALES-CARBONELL *et al.*, 2018). Classificação dos neurônios pelo padrão de atividade apresentado durante a tarefa. Foi utilizado para tal classificação o encaixe de funções polinomiais para calcular *tuning curves*.

Além disso, muitos destes estudos foram realizados tendo como região alvo o estriado dorsal e pouco é conhecido sobre a relação entre o estriado ventral, também conhecido como *nucleus accumbens* (NAc), e parâmetros motores. O NAc é normalmente relacionado à resposta a estímulos recompensadores ou aversivos (MCCULLOUGH et al. 1993; BOSCHEN et al., 2011; WENDLER et al. 2014). Entretanto, fortes evidências sugerem uma relação da atividade do NAc com a cinemática de aproximação à um lugar anteriormente recompensado e a proximidade do local em que é recompensado (SJULSON et al., 2018, MCGINTY et al., 2013). Desta forma, o NAc é um forte candidato para coordenar o comportamento de locomoção e aproximação relacionado às recompensas, pois diversos estudos mostraram que a inativação ou lesão do NAc bloqueia a expressão do comportamento de aproximação de

locais recompensados (BLAISS e JANAK, 2009; NICOLA, 2010). Além disso, o NAc recebe projeções diretas da formação hipocampal, área em que se localizam alguns dos neurônios essenciais para a navegação espacial (FLORESCO et al., 1997; FLORESCO et al., 2001), e apresenta projeções para o globo pálido ventral, região ligada ao controle de ações motoras (BERENDSE et al., 1992, ROOT et al., 2015). Trabalhos recentes têm confirmado a importância da interação entre a formação hipocampal e o NAc para o comportamento de preferência de lugar, demonstrando que o fortalecimento de sinapses entre esses neurônios aumentam a probabilidade de o animal passar mais tempo em determinado local (SJULSON, et al., 2018, LEGATES et al., 2018). A formação hipocampal, formada pelo giro denteado, hipocampo, *subiculum*, *presubiculum*, *parasubiculum* e córtex entorinal (AMARAL e LAVENEX, 2007), tem um papel importante no aprendizado e na formação de memórias declarativas. Além disso, estudos envolvendo place cells, descritas primeiramente por O'KEEFE e DOSTROVSKY (1971), revelaram o papel dos neurônios do hipocampo na orientação espacial. Estudos posteriores revelaram que outros tipos de neurônios na formação hipocampal também contribuem com outros tipos de informação espacial como velocidade, posicionamento nas bordas de um ambiente, direção da cabeça, por exemplo (MULLER *et al., 1987;* MULLER *et al.,* 1996; JUNG *et al.,* 1994; FRANK *et al.,* 2000; HAFTING *et al.,* 2005; SOLSTAD *et al.,* 2008; KROPFF *et al.,* 2015). Acredita-se que este conjunto de neurônios seriam responsáveis pelo sistema neural de orientação espacial, porém, não há evidências de que este sistema esteja diretamente ligado a áreas que controlam as atividades motoras (AMARAL e LAVENEX, 2007). Deste modo, este conjunto de neurônios poderia ser responsável pelo mapeamento e localização, entretanto, seria necessário o uso dessa informação para que a decisão de se locomover para um local em e não para outro seja tomada. Isto poderia implicar no recrutamento de estruturas cerebrais adicionais, potencialmente o NAc, o qual seria responsável pela integração das informações espaciais para realizar uma resposta motora de movimento para algum local em específico (DA CUNHA *et al.,* 2012).

Além disso, o NAc está diretamente relacionado ao comportamento de aproximação (onde o animal espera receber uma recompensa) e respostas recompensadas (MCGINTY *et al.,* 2013), pois também recebe projeções de regiões como a área tegmentar ventral (VTA, do inglês *ventral tegmental area*) (SADDORIS *et al.,* 2015), amígdala (MULDER *et al.,* 1998; FLORESCO *et al.*, 2001) e córtex pré-frontal (CPF) (TABER e FIBIGER, 1995; DEL ARCO e MORA, 2008; CRUZ *et al.,* 2015). Estas regiões codificam importantes informações relacionadas ao valor aversivo ou apetitivo de um comportamento, o que é crítico para a motivação para se aproximar ou evitar um determinado local (MULDER *et al.,* 1998; NAVRATILOVA e PORRECA, 2014; WENZEL *et al.,* 2015, FLORESCO, 2014) (Figura 3).

A VTA é conhecida por ser uma região do cérebro que possui muitos neurônios dopaminérgicos. A VTA é o principal componente da via mesolímbica, que distribui projeções dos neurônios dopaminérgicas para regiões como córtex pre-frontal, hipocampo, amígdala e NAc (IKEMOTO, 2010). A VTA é classicamente associada a processos de aprendizado associativo, motivação e atividade locomotora, dado a importância da dopamina para tais funções (ARNT et al., 1979, IKEMOTO et al., 1997, IKEMOTO 2010; COX e WITTEN, 2019). É bem estabelecido que esta região codifica informações relativas a recompensas e ao erro de predição (SCHULTZ, 1997). O erro de predição é uma medida da discrepância entre a recompensa esperada e a recompensa recebida. Além disso, o estudo recente de Engelhard e colaboradores (2019) mostrou que neurônios da VTA também codificam informações cinéticas relacionadas à locomoção como velocidade, aceleração e a outras informações espaciais, tais como a distância da recompensa durante uma tarefa que envolve aproximação de um local previamente recompensado. Isto implicaria que essas informações poderiam seriam transmitidas para o NAc por meio da liberação de DA, fortalecendo a hipótese de que o NAc possui um papel essencial para o controle da cinética da locomoção e codificação da distância da recompensa durante a aproximação. A amígdala por sua vez, apresenta um papel importante na resposta condicionada à estímulos ou dicas (MCLAUGHLIN e FLORESCO 2008; AMBROGGI et al., 2008). AMBROGGI e colaboradores (2008) demonstraram que a atividade dos neurônios do NAc mediada por DA é modulada pela

atividade da amígdala basolateral e que essa modulação teria efeitos diretos no comportamento de procura da recompensa. O papel da interação entre CPF e o NAc no comportamento de aproximação parece estar relacionada à avaliação do custo/benefício associado às respostas (HAUBER e SOMMER 2009), alta demanda de atenção (CHRISTAKOU et al. 2004), inibição comportamental e reaproximação de locais associados ao consumo de droga (CAMERON et al., 2018).

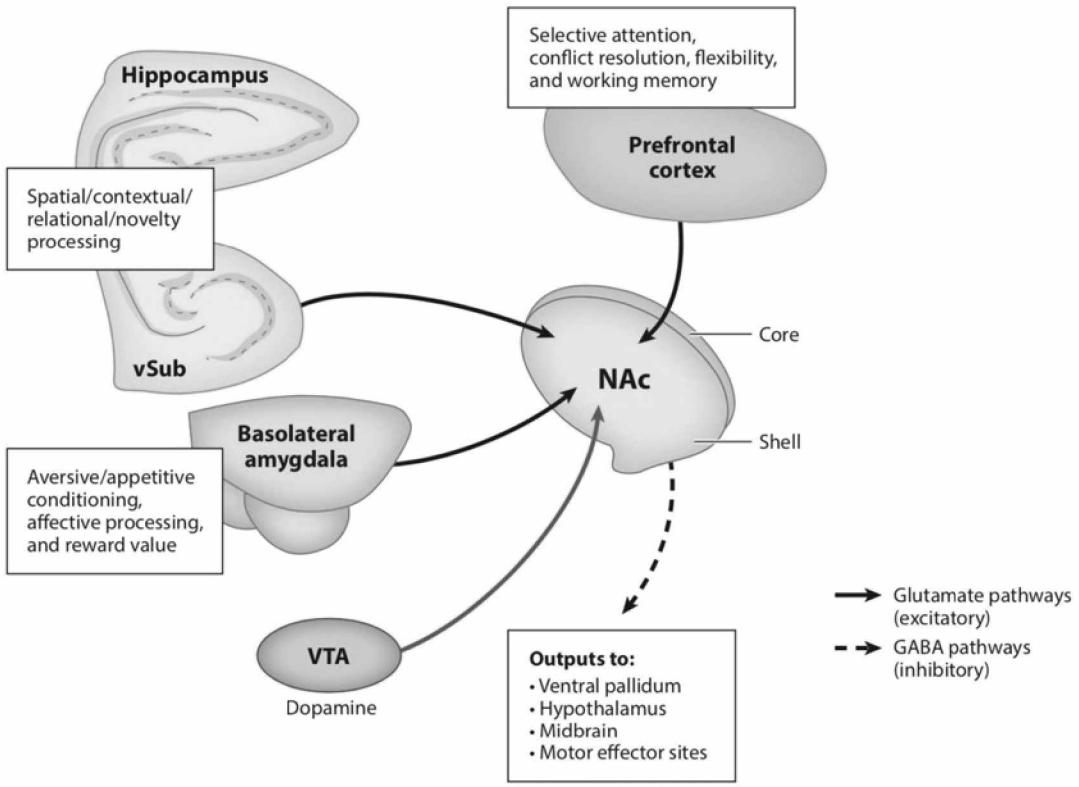


Figura 2. Esquema de *inputs e outputs* do NAc (Floresco, 2014). O NAc recebe projeções de regiões que fornecem diversas informações relevantes para o comportamento de aproximação. Além disso, o *outputs* do NAc vão para estruturas importantes para a execução de ações motoras.

Essas evidências sugerem que neurônios do NAc seriam responsáveis pela integração de informações espaciais e límbicas para a coordenação do comportamento de aproximação e, talvez, tomada de decisão sobre para onde se locomover. Entretanto se fazem necessárias evidências diretas se o NAc é responsável pela escolha de para qual lugar se locomover (FLORESCO, 2014).

Em relação à coordenação da ação de aproximação de locais previamente recompensados, trabalhos recentes demonstram a relação entre atividade dos neurônios do NAc e parâmetros cinéticos como a velocidade e a proximidade do local (MCGINTY et al., 2013), assim como foi observado no estriado dorsal (RUEDA-OROZCO et al., 2015; PANIGRAHI et al., 2015; YTRRI e DUDMAN et al., 2016; SALES-CARBONELL et al., 2018). Estes dados sugerem que o NAc também possui um papel importante na modulação da cinemática do comportamento de aproximação (MCGINTY et al., 2013, SJULSON et al., 2018). MCGINTY e colaboradores (2013) mostraram que, utilizando um protocolo experimental onde o animal (rato) era condicionado a associar a ocorrência de um som com a resposta de aproximação de um receptáculo para receber uma recompensa (solução de sacarose), 46% dos neurônios respondiam ao som, e interessantemente, a intensidade da ativação desses neurônios refletiam na velocidade média de aproximação do receptáculo onde o rato recebia a recompensa. Além disso, a proximidade do receptáculo também estava correlacionada positivamente com a maior ativação dos neurônios do NAc, demonstrando a codificação de informações relacionadas a distância do local da recompensa por estes neurônios. O estudo de MCGINTY *et al.* e também o trabalho de HOWE e colaboradores (2013) sugerem que a atividade dos neurônios do NAc pode ser modulada diretamente pela liberação de DA relacionada à proximidade do local onde é recebida a recompensa (local de aproximação). O estudo de HOWE e colaboradores (2013) mostra que o padrão de liberação de DA na região do estriado ventral está relacionada com a proximidade do local da recompensa. Estes resultados demonstram uma importante relação entre a atividade dos neurônios do NAc e os aspectos cinéticos e de proximidade do comportamento de aproximação.

Entretanto, alguns aspectos em relação à forma como essas informações são codificadas pelo NAc necessitam ser explorados: 1) Testar se os neurônios do NAc codificam informações relacionadas as seleção de ação (e.g. para qual lugar se locomover); 2) Testar se os neurônios do NAc também codificam aspectos cinéticos e de proximidade em uma tarefa de aproximação espontânea (sem condicionamento à dica) que envolve também a seleção de ações . Portanto, o objetivo deste trabalho é avaliar se e como a seleção de ações e/ou

o controle cinético do comportamento de aproximação estão sendo codificadas pelos neurônios do NAc durante o comportamento de aproximação que também envolve seleção de ação.

2. ARTIGO

Nucleus accumbens encodes speed and fraction of the distance to a reward

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Runing title: Speed and reward distance encoding in *nucleus accumbens*

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Conflict of interest statement: The authors declare no competing financial interests

Acknowledgments: This work was supported by CNPq grants 401077/2014-4, 432061/2018-5, 306855/2017-8, CAPES grant 23038.006539/2014-54, INNT grant 465346/2014-6, GACR grant 17-04047S and AZV grant 17-30833A.

- Number of pages: 28
- Number of figures: 7
- Number of tables: 0
- Number of multimedia and 3D models: 0
- Number of words for abstract: 175
- Number of words for introduction: 689
- Number of words for discussion: 1948

Authors contribution: D.L, C.D.C., M.A-R, and R.F. designed research; D.L., A.H.S., J.P., G.B., and L.N.P. performed research; A.H.S., D.L., G.B., J.P., S.M.N., C.D.C., and C.V.B. analyzed data; D.L., A.H.S., S.M.L., and C.D.C. wrote the paper.

Abstract

The *nucleus accumbens* is suggested to be a limbic-motor interface, and a key structure for reward seeking behavior. It is propose that the structure have a role in action selection (e.g. which place to go) and/or in the control of the motor response of the approach behavior (e.g. locomotion to the reward), however, which aspect or aspects are encoded by these neurons it is still poorly understood. Here we studied, in a spatial navigation task, the approach behavior involving action selection (choosing places to go), and evaluated the responses of single neurons. We identified neurons in the *nucleus n. accumbens* which encode speed and proximity to the reward, but no action selection evidences were found. We also observed that the encoding of speed and reward proximity was not homogeneous among the recorded neurons, suggesting the information is not encoded or does not arrive equally in the *n. accumbens*. Our findings favors the hypothesis in which these neurons have a role in motor control of reward approach, however, our analysis did not show any action selection related activity.

Introduction

Foraging behavior is fundamental for survival and it involves the selection of correct actions and their appropriate execution. The striatum is a midbrain structure well-known to be critical for both of these processes: action selection and motor control (Vinter and Gras et al., 1998; Isomura et al., 2013; Tacuapetla et al., 2016; Yttri and Dudman et al., 2016; Tsutsui-Kimura et al., 2017; Sales-Carbonell et al., 2018; Markowitz et al., 2018; Martiros et al., 2018). Previous studies proposed that the ventral striatum, also known as *nucleus accumbens* (NAc), as an important limbic-motor interface (Mogenson, 1980, 1993), which would evaluate and drive reward-related behavior. The neuroanatomy of the NAc supports this hypothesis as it receives mnemonic and emotional signals from regions such as the hippocampal formation and ventral tegmental area (VTA), and projects to a motor effector regions such as the ventral pallidum (Berendse et al., 1992; Floresco et al., 2001; Saddoris et al., 2015; LeGates et al., 2018; Sjulson et al., 2018; Trouchet et al., 2018; Engelhard et al., 2019).

Previous reports suggest that the NAc is especially involved in reward approach behavior (Blaiss and Janak, 2009; Nicola, 2010; McGinty et al., 2013). However, it is still unclear whether and how aspects of reward approach behavior like action selection and motor control (performance) are controlled by the NAc. In agreement with the hypothesis that the NAc invigorates locomotion (increase speed), electrophysiological recordings showed that the activity of NAc neurons of rats predicts the speed of a cued-evoked approach to the reward receptacle (McGinty et al., 2013; du Hoffman et al., 2014). Still, it was observed a correlation between the neuronal activation in NAc with the rat's proximity of the reward at the moment the approach response is evoked (McGinty et al., 2013; Morrison et al., 2014).

NAc dopamine seems to be critical to approach behavior because the blockade of dopamine receptors in the rat NAc reduced the number of reward approaches and slowed the initiation of the locomotion to the reward (Blaiss and Janak, 2009; Nicola, 2010; Saunders and Robinson, 2012; du Hoffman et al., 2014). It is possible that information about the kinematics and proximity of the reward is provided to NAc neurons by VTA dopamine neurons. Howe et al. (2013) showed that dopamine signals measured in the NAc with fast-scan cyclic voltammetry (FSCV) increased or decreased gradually as rats approached a distant reward place. In a recent study, Engelhard et al. (2019) used calcium imaging to record the single activity of more than 300 dopamine neurons from the VTA

as mice approached the reward place in a virtual reality T-maze task. They found that different cluster of dopamine neurons encoded different aspects of the task, such as decision making (turning right/left) the animal position, speed, acceleration and proximity to the reward place.

Together, these studies suggest that the aspects of action selection and motor performance of the cued-evoked reward approach response are modulated by the NAc. However, to the best of our knowledge no previous work tested whether the action-selection and kinematic aspects the reward approach behavior are encoded by single NAc neurons when the animal has to choose among several reward places and the locomotion response, instead of being evoked by a discriminative cue, is self-initiated.

Here, we tested this hypothesis by recording single unit activity in the NAc when overtrained rats choose to approach one out of 3 rewarded arms of a radial maze. Part of the animals was trained to find the same amount of reward in the 3 rewarded arms and the other part was trained to find different amount and probability of rewards in the 3 rewarded arms. Our results suggest that different subgroups of NAc neurons encode speed and reward proximity in different proportions, with subgroups of neurons mostly encoding speed or reward proximity, however, a few encoding both similarly. Thus, our finding reinforces the hypothesis that the NAc modulates motor performance of appetitive approach independent of this behavior to be self-initiated or cue-evoked and independent of the reward to be available in only in one place (no choice) or the animal to have the choice to choose among different reward places.

Methods

Subjects

We used adult male Wistar rats that were 3 months old at the beginning of the experiment. The rats came from the breeding colony of the Federal University of Parana and were housed in groups of 4 per cage during behavioral training and individually after surgery. The rats were maintained in a temperature-controlled room (22 ± 2 °C) with a 12-hr light/dark cycle (lights on at 7:00 am). Access to water was restricted to one hour per day, and access to food was restricted to maintain the rats' body weight at 90% of their free-feeding weight (290-340 g). All experimental procedures were in agreement with the Brazilian and International legislation for animal care (Law N° 11.794, 8 October 2008; EC Council Directive, 24 November 1986; 86/609/EEC). The project was approved by the Animal Care and Use Committee of the Federal University of Parana and efforts were made to minimize the number of animals used, and their suffering and discomfort during the experimental procedures.

Apparatus

We used an eight-arm radial maze for behavioral training. Each arm (62 cm x 13 cm) and the central platform (30 cm diameter) were elevated 60 cm above the floor. The walls of all arms were 5 cm high. The entire maze was made of stainless steel and its surface was covered with black contact paper. One or four drops (25 µl per drop) of chocolate milk were delivered at the ends of rewarded arms before each trial. White curtains were installed around the maze; several salient visual cues (black felt shapes) were attached to it and remained in the same locations throughout training and experiments. Four light bulbs (15W) were spaced equally on a metal frame (70 x 70 cm) above the center of the maze. This metal frame also served as a support for a motorized commutator (Plexon, USA), a camera connected to the main computer, and for an amplifier (Plexon, USA). An OmniPlex D Neural Data Acquisition system for up to 64 channels (Plexon, USA) was located in the same room. Metal mesh was installed on the walls of the experimental room and grounded to form a Faraday cage.

Behavioral procedures

All rats were handled (5 min/day) by the experimenter for 3 consecutive days before the start of behavioral pre-training. During pre-training, the rats were habituated to the maze

and reward (chocolate milk) by placing them on the end of one arm and letting them drink the chocolate milk from the reward receptacle.

After the pre-training phase, the rats were trained to collect drops of chocolate milk consistently located at the ends of the same three arms of an eight-arm radial arm maze. In the *same reward group* (n = 2), all the reward arms contained 4 drops of chocolate milk (100 µl) in 100% of trials. In the *different reward group* (n = 3), one of the reward arms contained four drops (100 µl) of chocolate milk in 100 % of trials (high reward, H arm), another reward arm contained four drops (100 µl) in 66.7% of trials (medium reward, M arm), and the last reward arm contained one drop (25 µl) in 100% of trials (low reward, L arm). Another arm was consistently used as a resting platform, where the rats were placed and restricted between trials. The other four arms were used as starting positions (Fig. 1A). The positions of the baited arms were counterbalanced among rats and were chosen in a pseudorandom manner so that there were never two adjacent arms baited. The rats underwent nine trials per day. One of the starting positions was used three times and the other two times in a pseudorandom order. Each trial finished when all rewards were collected or after 5 min elapsed. The rats were trained five days per week until they reached the following criteria in three consecutive training days: a) no more than 20% reference memory errors (entering a non-rewarded arm); b) the 4-drop (100% probability) reward arm may be the last choice in no more than 20% of trials; and c) the 1-drop reward arm may be the first choice in no more than 20% of trials. Rats took 40 to 50 training days to achieve these criteria.

Afterwards, the rats underwent a surgery to implant the electrode array. After recovery, they were re-trained to the pre-surgery level of performance, which took approximately seven days. During these re-training sessions and the following test sessions, 12 trials per session were carried out.

Surgery

The rats were anesthetized with isoflurane (2%), placed on a stereotaxic frame (David Kopf, USA) and 2x4 stereotrode arrays (17 µm Ni-Chrome wire with insulation) installed in a custom microdrive were chronically implanted unilaterally above NAc-shell in the following coordinates: AP +1.2 to +2.2 mm from bregma, ML +0.6 to +1.0 mm from midline, DV -5.0 mm from dura. The coordinates were determined according to Paxinos and Watson atlas (2007) and adjusted according to our pilot surgeries. Six anchoring stainless steel screws were placed in the skull to attach the implant using acrylic dental

cement. One of the screws also served as a ground. After surgery, enrofloxacin (20 mg/kg; i.m.) was administrated and we applied a gel containing neomycin to the tissue around the implant, and ibuprofen (50mg/500ml) was added into the drinking water for the 3 following days. The rats were allowed to recover for 7 days after the surgical procedures.

Electrophysiological recording

After one week of recovery, the stereotrodes were lowered in 40 μm increments (up to 240 μm/day) until single-unit activity was reliably detected and isolated. All stereotrodes were previously gold-plated with a nanoZ impedance tester (Plexon, USA) to impedances between 100-300 kOhms. To be accepted for recording, cell signals had to be at least three times greater than the background activity. Two light-emitting diodes attached to the 16-channel headstage (20x gain) signaled the position of the animal and allowed video tracking with the Cineplex system (Plexon, USA). Neural activity was amplified (1000 x), filtered (300-6000 Hz) and sampled at 40 kHz with the OmniPlex D Neural Data Acquisition system (Plexon, USA) to record single spikes.

Histology

After completion of recording procedures, microlesions (12 μA, negative current, 30 s duration) were made at the tips of all stereotrodes using the nanoZ (Plexon, USA). Subsequently, the rats received ketamine (75 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.) and they were transcardially perfused with 300 ml saline followed by 300 ml 4% paraformaldehyde. The brains were removed and 40 μm slices were processed for cresyl violet staining. The final location of all electrodes was verified.

Spike sorting

All spikes were manually clustered offline into putative cells with Offline Sorter software (Plexon, USA). Clusters were based on waveform properties identified through principal component analysis. Inter-spike interval histograms were calculated, and those clusters with similar waveform shape that showed a clearly recognizable refractory period (> 1.5 ms) were considered as originating from a single neuron.

Neuronal activity analysis

A test session consisted of 12 trials. In each trial, the rats usually approached the reward areas of the three rewarded arms only once. Each approach was defined as a run – when the rat ran from the distal end of one arm towards the reward area of a rewarded arm. Because trained rats performed few visits to non-rewarded arms (Figure. 1B), only data from approaches to rewarded arms were analyzed. The data window analyzed for each run extended from 1 s before locomotion onset to 1 s after locomotion end, respectively. Because the runs were stereotyped in general (as in the example in the Figure 1C), locomotion onset was defined as the first point in which the velocity of the animal reached a threshold of 20 cm/s before the peak speed of the run (Figure 1D). Locomotion end was set as the first point at which the speed dropped below a threshold of 10 cm/s (see Extended Figure 1-1). We restricted our analysis to approaches to the reward defined by an efficiency criterion, which was calculated as the ratio of the distance traveled in the run to the length of the ideal path (from the end of one arm to another). Runs with high efficiency (rate<0.8) or low efficiency (rate>1.2) were discarded to avoid runs in which the animal did not start the run close to the beginning of one arm or deviated substantially from a direct approach to the end of the reward arm.

For some analyses, neuronal data was aligned to each of the following events: locomotion onset, locomotion end, entering the center zone, entering the target arm, and reaching peak speed. Data were divided into 200 ms bins. The data aligned to these events were averaged across all reward approaches of the session and transformed to z-score. Then, the neuron's responsiveness to each event was evaluated. For locomotion onset and locomotion end, the responsiveness of the neurons was tested for the periods of 1.5 seconds after the event and 1.5 seconds before the event, respectively. For data entering the center zone, entering in the target arm times, and reaching the speed peak, the analysis was done for the periods of 0.75 s before and 0.75 s after the event. The neuronal response of each bin was considered significant if the firing rate exceeded the 95% confidence interval ($Iz - score I > 1.98$).

For the neuronal data normalized by time, independent of the run duration, each run was divided into 60 bins and frequencies of spikes during each fraction were averaged by the number of runs, smoothed and transformed in z-score based on the neuronal activity during only the approaches to rewarded arms.

Generalized linear model (GLM)

The goal of this multiple regression analysis was to evaluate which performance-related parameters can be used to predict neuronal firing rate. A generalized linear model (GLM) was calculated with the firing rate (spikes/bin) as the dependent variable and 12 parameters as predictors (independent variables). Seven predictors were continuous variables: speed, acceleration, fraction of the run (fraction of the total distance traveled during the reward approach; calculated as the rate between distance traveled and total distance traveled until the end of the run), angle-center (angle formed by the position of the rat and the center of the maze), head direction (direction of the head), head movement (ipsilateral or contralateral movements), center distance (distance of the animal relatively to the center of the maze). Three other predictors were whole-run values (i.e., they were the same for all the bins in a given run): max speed (maximum speed during the reward approach), run duration (duration of the reward approach), trial (number of the trial). Two other predictions were categorical and used to determine which arm the animal was approaching: arm2 (1 if the rat choose to enter arm 2, 0 if he chooses arm 1 or arm 3); and arm3 (1 if the rat enters arm3, 0 if he enters arms 1 or 2). The model considers the rat to have entered arm 1 when both arm 2 and arm 3 are 0. Pearson correlations (r) between the predictor variables were calculated and, for the ones that showed r higher than 0.8, one of them was excluded (e.g. time and fraction of the run; Extended Figure 4-1).

Continuous variables were extracted from the coordinates tracked in the recorded videos. The rat's locations were tracked at 30 frames per second. However, to match the neuronal data that were binned at 100 ms, an interval of frames was used (3 frames = 100 ms interval). Firing rate, speed and acceleration were smoothed by a sliding average window of 300 ms (Kropft et al., 2015; Rueda-Orozco and Robbe 2015). The β values were calculated with the MATLAB glmfit function. The multiple regression assumed a normal distribution for the data and the identity link function was used to estimate the β value for each parameter.

The GLM followed the equation:

$$Y_{(t)} = \beta o + \beta_1 x_{1(t)} + \beta_2 x_{2(t)} \ldots + \beta_n x_{n(t)} + \varepsilon$$

In which Y_t is the predicted firing rate at time t, β_n is the weight of the predictor n, $x_{n(t)}$ is the value of the predictor n at time t, and ε is the error term. For each neuron, the model was trained and tested by the fivefold cross validation method (Engelhard et

al., 2019) as follows. The data from all the runs (speed, acceleration, firing rate, etc) were divided into 5 parts with the same number of runs. The runs used in each part were randomly selected. The model was trained and tested five times with 4/5 of the total data, and the ability of the model to predict the remaining 1/5 of the data was assessed by correlating the predicted data to the actual data (R^2 of the correlation). Always, a different combination of the data was used as training and test data (Extended Figure 4-2).

The mean of the 5 R^2*100 values obtained from the fivefold validation was considered the mean percentage of variance explained by the full model (FM, model with all predictors included). The neurons were classified as "significantly explained" and "not significantly explained" by the FM as follows. The significance of the model was evaluated by comparing the variance explained by the neuron's FM to the distribution of variance explained by models trained with shuffled neuronal data (Figure 4; 100 shuffled models per neuron; n = 66). Neurons for which the model explained the actual data better than the 99th percentile of the shuffled models were considered significantly explained by the encoding model.

Contribution of each predictor to the GLM

To evaluate which variables had a significant contribution to the full model (FM), only neurons significantly explained by the FM were analyzed. The variance explained by the FM and the FM with one variable removed was compared across the population with a paired t-test and Holm-Sidak correction for multiple comparisons (Figure 5). To calculate the contribution of each of the variables (var) to the FM, the FM was compared to the full model less the selected variable (FM – var). The percent of the variance of the FM explained by each variable was calculated as:

$$C = \left(1 - R^2_{FM|FM-var}\right) * 100$$

$$C = \left(1 - R^2_{FM|FM-var}\right) * 100$$

In which C is the percent of the contribution of the variable to the FM and $R^2_{FM|FM-var}$ is the R^2 of the correlation between the FM and the FM-var.

Correlation maxima

To test if changes in firing rate during the runs preceded changes in speed or fraction of the run, the data for neuronal and predictor variables from all the runs were compiled into

a single time series vector, and the speed and fraction data of all of the runs were systematically shifted forward and backward by bins of 100 ms. The correlation was recalculated for each time shift and the correlation maxima lag was defined as the time shift with the highest correlation. Then, the average of the best fit time shift was calculated for each population (e.g. +Speed). The tuning curves shown in Figure 6 were calculated by averaging the normalized correlation across the populations of each time shift.

Experimental Design and Statistical Analysis

Details of statistical analysis of responsiveness to the events and significance of the encoding model are detailed in the Methods section. The paired t-test was used to evaluate the significance of the contribution of each predictor tested. The Holm-Sidak method was used to correct the p-values for multiple comparisons.

Code Accessibility

The custom MATLAB code used for the data analysis of this study will be made available from the corresponding author upon reasonable request.

Results

Behavioral task and performance

During the recording sessions, rats showed a standardized temporal change in velocity across the approach runs to collect the rewards in the 3 baited arms (as in Figure 1D). This stereotyped behavior made possible the analysis of individual reward receptacle approaches (runs) (Figure 1C). Because approaches to the non-rewarded arms was infrequent, only runs towards the rewarded arms were analyzed. In addition, an efficiency criterion was used to exclude non-stereotyped runs (see Methods). The distribution of the duration of the valid reward approaches is shown in Figure 1E (mean $\pm$ SD = 3.87 s $\pm$ 0.82).

Neuronal activity of NAc cells is modulated during, before and after kinematic and behaviorally relevant events

The activity of 66 neurons was recorded while the rats (n=5) collected the rewards at the end of the rewarded arms. Most of the recorded neurons were located in the NAc shell

(Figure 2). To test if these neurons were responsive to kinematic variables and/or events related to decision making, we first aligned the neuronal data to locomotion onset, locomotion end, peak velocity, entrance into the center zone of the radial maze, and entrance into the target arm. The locomotion onset alignment shows that, in general, the neuronal modulations starts a few miliseconds before the onset, however stronger neuronal responses happens after it. On the other hand, the modulations observed at locomotion end were present before the event. For the other alignments (entrance in the center zone and target arm, and the peak of speed), the firing responses tended to occur both before and after the event.

The significance of the firing responses was analyzed by comparing the firing in specific windows before, during and after the event (see Methods). The activity of all recorded neurons aligned to the 5 events is illustrated in Figure 3A. The heat maps are sorted by the firing rate in a 1.5 s window where the response was analyzed. Of the 66 neurons, 26 neurons showed significant responses when aligned by locomotion onset time; 25 when aligned by peak speed; 20 when aligned by locomotion end; 15 when aligned by entrance into the center zone, and 9 when aligned by entrance into the target arm (Figure 3B). In total, nearly 67% (44 units) of the neurons presented a significant response to at least one of these events (Figure 3C). Thus, these results suggest the NAc neurons were modulated by kinematic and/or behavioral properties of the task.

Behavioral and kinematic variables significantly predict neuronal activity

To evaluate and better distinguish the kinematic and/or behavioral parameters/events responsible for the responses of recorded neurons, we used an encoding model calculated using a generalized linear model (GLM; Engelhard et al., 2019; Figure 4A). Kinematic and behavioral variables were used as predictors of the GLM while the firing rate was defined as the dependent variable (for details on the predictors of the model, see Methods and Extended Figure 4-1).- For each neuron, we first obtained β values from running the GLM on a randomly-chosen 4/5 of the data. We then used the resulting model to predict the firing rates in the remaining 1/5 of the data (see Methods). Figure 4B shows how well the model predicted the firing rates of 3 representative neurons across 5 different maze runs. The R^2 between the actual and the predicted data was calculated to quantify the variance explained by the model. The percentage of variance explained by the encoding model was calculated by multiplying the R^2 by 100. To evaluate the GLMs, we compared the percentage of variance explained by the encoding models to the power of prediction

of encoding models trained with randomly shuffled firing rate data (100 shuffles per recorded neuron). In cases where the encoding model explained the variance in firing rate better than the 99th percentile of the shuffled data models, the neuron's encoding model was considered significant. The firing rate of nearly 62% (42 units) of the recorded neurons were significantly explained by their encoding models (Figures 4C and 4D), suggesting that more than half of the recorded neurons are modulated by kinematic and/or behavioral variables during the approach behavior.

To better evaluate the predictive power of each of the independent variables to the encoding model, the percentage variance explained by the full model (FM; encoding model with all variables included) was compared to the percentage variance explained by the FM without the selected variable (FM-var). Only the 42 units for which the encoding model significantly predicted the change in the firing rate were included in this analysis. Representative illustrations of the effects of removing a variable from the full model are shown in Figure 5A. As shown, the variables that contributed the most to the encoding models were the speed of the animal and the fraction of the run traveled. To evaluate this effect, we calculated the mean (across the population) of the percentage of variance explained by each predictor, and then compared these means for the FM and FM-var for each variable using paired t-tests corrected for multiple comparisons by the Holm-Sidak method (n=42; Speed $p=7x10^{-5}$, Frac run $p=4.1x10^{-6}$; Figures 5B). These results suggest that across the population of the recorded neurons the variables of the FM that contribute the most as predictors of firing rate variance are the speed and the fraction of the run. Figures 5C and 5D illustrate the effects of removing both the speed and fraction of the run variables on the discrepancy between the firing rate predicted by the models and the real data (n=42; the significance of paired t-test after Holm-Sidak method for multiple comparisons: $p=4x10^{-6}$).

NAc neurons code speed and fraction of the run

To test whether the contributions of speed and fraction of the run were similar across neurons, we used a simpler model (SM) using only speed and fraction of the run as predictor variables. Here, only neurons significantly explained by the SM were analyzed (SM predicted the actual data better than the 99th percentile of the surrogated models; n=38). From these 38 neurons, only 2 neurons were not included in the 42 that were significant with the FM.

Using the SM, we evaluated the contribution of each variable to the SM by comparing the variance explained by the SM and the SM without one of the predictor variables (speed or fraction of the run). Because many neurons exhibited a substantially greater contribution of one predictor over the other (Fig. 6A and 6B), we next tested if there was a distinct separation between these neurons activity patterns. First, based on the contribution of each variable we separated the neurons into two groups: Speed- and Fraction-related cells. Only neurons with the contribution of speed or fraction of the run higher than 20% over the other variable were included in these groups (e.g. speed contribution 60% vs fraction contribution 40%, but not speed 50% vs fraction 35%). Next, these two groups were subdivided into positively and negatively related to the variable: +Speed, -Speed, +Fraction, and -Fraction. The positive or negative correlation was determined by the sign of the SM β value. The averaged activity of these 4 classes of neurons are shown in Figure 5C. The neuronal activity presented was normalized for the time (more details in Methods) in order to visualize the responses of these neurons to the locomotion onset and the locomotion end in the same graph. The 4 groups presented distinguishable neuronal activity patterns (Figure 6C): +Speed neurons are characterized by a prolonged activation beginning at locomotion onset and inhibition beginning just prior to locomotion end; -Speed neurons showed the inverse pattern; +Fraction and -Fraction neurons also revealed opposite patterns in which firing gradually increased or decreased from locomotion onset to offset, respectively. The firing of +Fraction and -Fraction neurons typically crossed a Z score of 0 (representing no difference from the mean firing rate of the neuron during runs) near the middle of the run. When comparing the changes in speed and changes in neuronal activity of +Speed and -Speed neurons, it is clear that the changes have a high correlation with each other (Figure 7A). The +Fraction and -Fraction neurons presented modulations of the activity that can be correlated with the proximity to the reward. However, the activation of the -Fraction neurons at the beginning of the approaches could be related only with the initiation of the approach. The unclassified neurons did demonstrate activity patterns that could be attributed to the combination of both information, speed and distance to the reward (as shown in example neuron in the center of Figure 5A; Extended Figure 5-1C).

Changes in neuronal activitity follows or succed the changes of velocity and proximity to reward

If a change in firing rate precedes a change in a behavioral parameter such as speed, then the firing could, in principle, causally influence the measured behavior. On the other hand, changes in firing that accompany or succeed changes in behavior cannot cause the behavioral change. To test whether changes in firing rate precede or succeed changes in speed (for speed related cells) and the fraction of the run (for fraction related cells), we systematically shifted the behavioral data by bins of 100 ms forward and backwards and correlated the shifted behavioral and firing rate data. The time shift for which the neuron presented the highest (or lowest for negative correlations) Pearson's correlation coefficient was considered the |r| maxima lag. Across the +Speed population, the mean correlation maxima lag was -209 ms $\pm$ 117 (mean $\pm$ SEM), meaning that changes in firing preceded changes in speed by roughly 200 ms, however, an one sample t-test showed no significance when compare to 0 ($p = 0.10$). -Speed neurons the |r| maxima lag was 40 ms $\pm$ 51, $p = 0.48$, also showing no significant difference .For the fraction related neurons, the mean correlation maxima lag was: +Fraction = -43 ms $\pm$ 89, $p= 0.65$; -Fraction = 162 ms $\pm$ 50, $p = 0.01$. Although +Speed, +Fraction, and -Speed neurons showed a negative or positive maxima lag average across the group, no significance was found, and these results suggest that most of the changes in neuronal activity follows the changes the in speed or fraction of the run, putatively excluding the hypothesis that this neurons are causing the changes in the behavior (e.g. increasing the speed of the animal), however, as most of the +Speed neurons showed better correlation with negative shifts (Figure 7B), we can argue that this causality it is still possible . For -Fraction in which the average maxima lag was positive and significantly different of 0 (zero), the result suggest the neuronal activity of these neurons succeed the changes in the proximity to the reward. Together, these results suggest the activity of +Speed, -Speed, and +Fraction neurons follows the changes in speed/fraction of the run, which could be modulating the responses, but unlikely to be causing increases in speed, for example. Including the -Fraction group, the neuronal responses of all groups could work as reporters of the ongoing behavior to other regions of the brain.

Discussion

The neural encoding of the kinematics and proximity to a target place during the approach behavior is still not well understood. The ventral striatum is a candidate structure for coding such parameters. Our results showed that ~67% of the recorded neurons in the

NAc respond to kinematic variables or behavioral events (e.g. locomotion onset and entering the center zone) in the radial arm maze spatial task. The GLM encoding model showed that these neurons encode mostly two of the tested parameters: speed and fraction of distance to the reward (fraction of the run). However, variables related to action selection (e.g. target arm location) did not contributed significantly to the GLM, which suggest no action selection encoding by the NAc neurons. We observed in a subpopulation of neurons that only encode these variables (speed and reward distance), in which these neurons were encoding these parameters heteregenously (e.g. a neuron enconding mostly speed). These neurons with a major encoding of one variable over the other presented distinct patterns of activity. These two groups could also be divided into subgroups in which the variable was negatively or positively correlated with the firing rate. Thus, the neurons that could be classified in the four subgroups (+Speed, -Speed, +Fraction, and -Fraction) showing distinct neuronal activity patterns in which the positively and negatively correlated groups demonstrated opposite dynamics. The neurons that we could not classify because their contributions of both variables (speed and distance fraction) to the encoding model were too similar, presented an activity that could be attributed to the complementary contribution of both variables to the encoding of these neurons (e.g. middle column in Figure 5A). The contributions of each variable showed a gradient across the neurons and did not completely divide the cells into populations of speed and fraction neurons. Thus, we suggest that the patterns observed in the classified and unclassified groups could be a result of heterogeneous way these parameters are encoded in NAc. These neurons might receive or encode different proportions of speed and distance information, and the speed and fraction related cells described here, representing the cells that receive inputs mostly related to speed and distance to a target, respectively. This type of architecture could serve as a putative mechanism for fine signaling or control of the locomotion along with the approach behavior. Such neurons could signal and/or cause the changes in the kinematics during movement.

Previous studies strongly support our findings of kinematic and proximity encoding in the striatum (van der Meer and Redish, 2009; McGinty et al., 2013; Howe et al., 2013; Isomura et al. 2013, Panigrahi et al., 2015; Rueda-Orozco and Robbe, 2015; Yttri and Dudman, 2016; Carbonell et al., 2018; Sjulson et al., 2018). The dorsal striatum neurons modulation by paws movement was observed in electrophysiology and optogenetic experiments (Carelli and West, 1991; Isomura et al., 2013; Panigrahi et al.,

2015; 5; Yttri and Dudman, 2016). Other studies reported correlations of speed with neuronal activity in dorsal striatum while rats were running in a treadmill (Rueda-Orozco and Robbe, 2015; Carbonell et al., 2018). Specifically in the ventral striatum, there is evidence that disruption of dopamine transmission in the NAc slows the approach behavior (Salamone et al., 2007; Nicola, 2010; du Hoffman and Nicola, 2014), and NAc neurons response reflects the vigor/speed of the approach to the reward (van der Meer and Redish, 2009; McGinty et al., 2013). Those findings strong support the hypothesis that the NAc neurons also encode the cinematic aspects of approach behavior. In addition, neuronal activity of the VTA (which innervates NAc) and DA release in the striatum presented correlation with kinematic parameters and reward proximity (Howe et al., 2013; du Hoffman and Nicola, 2014; Engelhard et al., 2019). Together, these results reinforce the hypothesis that the NAc encodes speed and proximity to the reward. Our encoding model suggests that both speed and proximity to the reward were encoded by the NAc. However, any encoding related to approaching a specific arm, reward value or head direction were not observed. Although the selection of target places could be encoded by a structure which receives spatial information from the hippocampal formation as the NAc (Floresco et al., 1997; LeGates et al., 2018; Sjulson et al., 2018; Trouchet et al., 2019), our results, in agreement with previous findings (Floresco et al., 2001; Howe et al. 2013), suggest that spatial information arrives in NAc is potentially translated into a reward proximity code, instead of determining the place to approach.

The NAc receives direct glutamatergic projections from hippocampal formation (Floresco et al., 1997; Humphries and Prescott, 2010; LeGates et al., 2018; Sjulson et al., 2018), a brain region crucial for spatial navigation (Packard and McGaugh, 1996; Floresco et al., 1997; Trouchet et al., 2019; Morris et al., 1982), but it is the DA release from VTA which seems to be the responsible for signalizing the reward place proximity (Howe et al., 2013). Previous study supports a hypothesis that the spatial information from the hippocampal formation is sent indirectly via NAc to the VTA dopaminergic neurons, possibly, translating it into proximity information (Floresco et al., 2001). Floresco and colleagues (2001) showed that pharmacological activations of ventral subiculum (a component of the hippocampal formation) modulates the activity of VTA dopaminergic neurons. This activation was abolished by infusions of a glutamate antagonist in the NAc, which suggest the NAc mediates the indirect effect of ventral subiculum on DA release (Floresco et al., 2001). Putatively, the NAc could act as the "translator" of spatial to proximity information. In addition, as this proximity signal

coded by DA neurons would be sent back to NAc (Howe et al., 2013), in which DA is known to affect approach behavior (Salamone et al., 2007; Nicola, 2010; Saunders and Robinson, 2012; du Hoffman and Nicola, 2014). This suggests the DA signal mediated indirectly by the ventral subiculum (spatial information) could also affect the kinematics of the approach behavior. These results potentially explains how the NAc could be involved in encoding both information, target proximity and speed.

In addition, we showed for the first time, to the best of our knowledge, that NAc neurons encode speed and proximity information heterogeneously across the neurons. McGinty and colleagues (2013) have shown that 46% of the neurons they recorded in NAc encoded vigor (speed) and reward receptacle proximity, however, their data presented that the activation of these neurons was correlated to the receptacle proximity and the vigor of the response, simultaneously. Our results showed that 57% of the recorded neurons in NAc were encoding these parameters, though, in different proportions for each neuron (e.g. a neuron mostly encoding speed), in which we could classify them depending on the contribution of each parameter to the encoding model into four categories (+Speed, -Speed, +Fraction and -Fraction). In addition, in contrast to the phasic neuronal response to a cue signaling reward availability preceding the approach initiation previous reported (McGinty et al., 2013), the neuronal encoding observed in our task seems to be moment-to-moment related (gradual changes in firing rate along the approach behavior). This suggest an role of NAc in the modulation of locomotion in both, cue-evoked and spontaneous approach. The causal effect over the control of locomotion is not totally supported by our results, because of the changes in neuronal activity across the population followed the proximity to the reward or the changes in speed instead of preceding it, however, most of +Speed neurons presented better correlation with negatively shifted data, in which suggests the changes in firing rate preceded the changes in speed for these neurons. We propose the activity of these neurons could act as source of information that could modulate motor responses but not causing it.

The groups of neurons with activation positively or negatively correlated to speed showed opposite dynamics (e.g. +Speed vs. -Speed; Figure 5C). We can speculate that the neurons with these two opposite patterns could be part of distinct striatal pathways (direct and indirect). Previous studies showed that activation of the direct pathway promotes locomotion while the opposite happens when the indirect pathway is stimulated (Bateup et al., 2010; Kravitz et al., 2010). Following this idea, the -Speed and +Speed neurons could potentially correspond to the indirect and direct pathways, respectively.

For the +Fraction and –Fraction neurons, it is not clear how the activity of these neurons could affect locomotion (if so). Thus, it is difficult to define whether and how these neurons would integrate these pathways.

We propose three potential explanations for the differences in the NAc neuronal encoding observed in our approach task (moment-to-moment response starting on locomotion onset) and in the cue-evoked approach task in McGinty and colleagues work (instantaneous response starting after cue and before locomotion onset): 1) As the availability of reward in McGinty and colleagues study is signalized by an instaneous auditory cue, the cue-evoked neuronal response possibly encodes future speed and proximity also phasically, while the neuronal response in spontaneous approach (as in our task) may encode the information subsequently moment-to-moment; 2) McGinty's experiments did not require the rats to make long approaches as the conditioning box is relatively small in comparison with our radial arm maze (distance from one radial maze arm to another = ~160 cm; conditioning box = 40 x 40 cm). Thus, the gradual modulation during the goal approach in the conditioning box could not be measured as the goal was usually located in a very short distance (~20 cm); 3) In McGinty's task, the reward receptacle was fixed and visible at the same position and no navigation based on cognitive maps was required. Thus, after the rat is familiarized with the box and the trajectories to the receptacle, to compute proximity of the target place and the appropriate response before starting the approach could be simpler (one phasic activation) than in our experiments (not visible reward/receptacle and cognitive based navigation with multiple reward receptacles). This is supported by classical study from Morris and colleagues (1982) where is shown that lesions in hippocampus impaired the rat's hability to find a hidden plataform (spatial navigation required), but not when the plataform was visible and a cognitive map was not necessary. As metioned above, the NAc (especially the shell) receives direct projections from the hippocampal formation, which is essential for spatial appetitive navigation (Trouchet et al., 2019).

The fact that the recorded neurons in McGinty and colleagues were placed mainly in NAc core and not in NAc shell as in our study was not considered a significant difference because van der Meer and Redish (2009) also observed neuronal modulations in the ventral striatum (mostly in NAc core neurons) similar to the ones observed in our data. In the Meer and Redish study the neurons also presented modulations of the activity along approach behavior with some neurons showing speed-related activity. Because our task does not involve cue-evoked behavioral response or fixed target approach or shorter

approaches, we could not verify whether these differences in encoding of NAc neurons are directly related to the differences in the protocol.

In conclusion, our findings support the hypothesis that also during spontaneous approach behavior involving spatial navigation the NAc neurons encode speed and the reward proximity, however, the proportion of encoding of each parameter was different across the population and different patterns of neuronal modulations during the approach behavior were observed. These patterns were different from the previous report from McGinty and colleagues, though, differences in the specific type of approach behavior can explain the different modulations. Moreover, further investigation is necessary to better understand how the same NAc neurons respond to different approaching tasks (e.g. cue-evoked and spontaneous, or target directed and cognitive based). In addition, optotagging techniques will be useful to determine whether the classes of neurons observed in the present study belongs to the direct or indirect pathways, which could help the understanding of how specific neurons can be influencing the motor control during the approach behavior.

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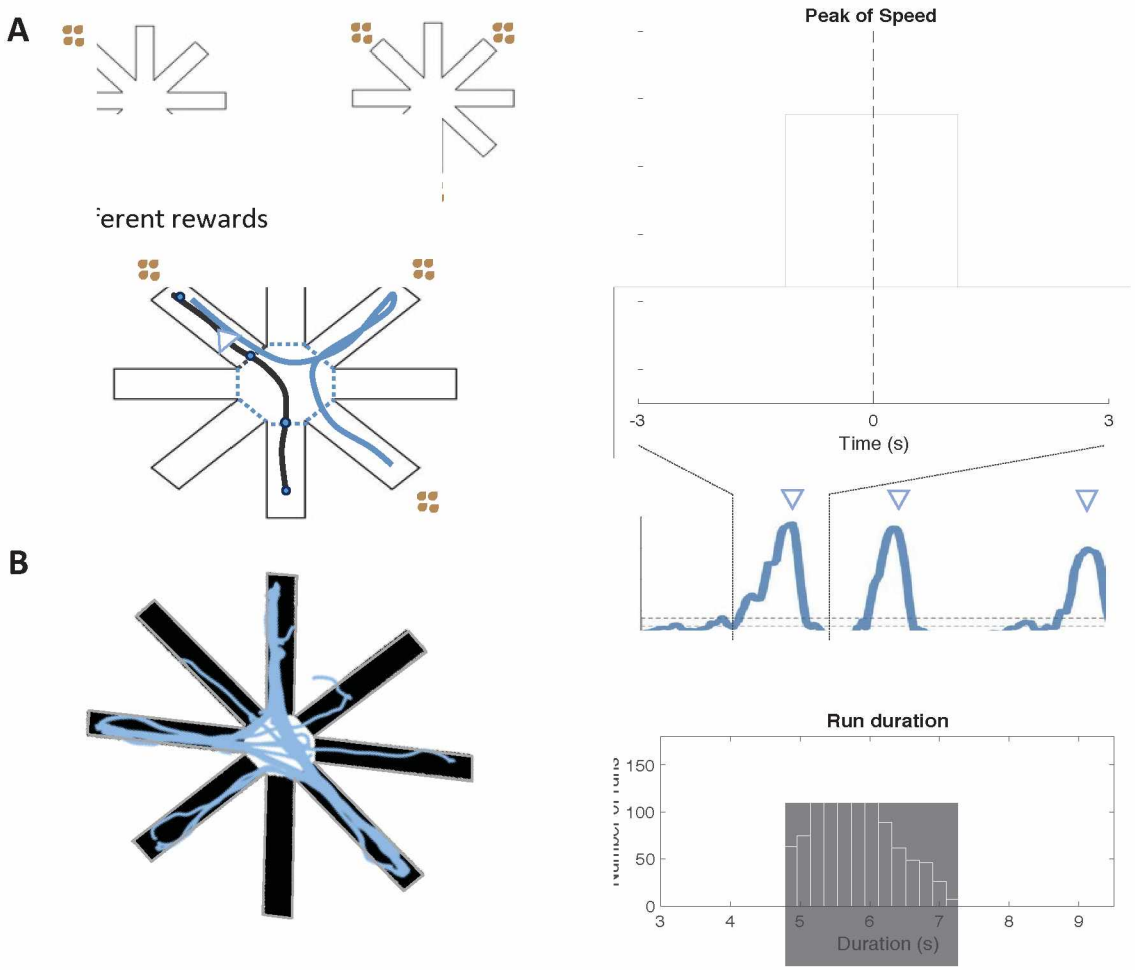


Figure 1: *Stereotyped kinematic behavior of over trained rats in the radial arm maze task.*

A, Illustration of the behavioral task in an eight arm radial maze. Each trial began with the rat been placed one of the non-rewarded arms and finished when the animal have collected all three rewards. Two rats received 4 (100 μl) chocolate milk drops in the end of the 3 baited arms. Three other rats received 1 (25 μl) drop of same reward in the end of one arm, 4 (100 μl) drops in the end of another arm, 4 (100 μl) drops in the another arm, but only 66% of the times. Each trial was divided in rewarded arms approaches (runs) for analysis of the neuronal activity. The data analyzed in each approach was one second before the locomotion onset and ands one second after the rat arrives the end (reward area) of the target arm. **B,** A few superimposed tracks of the rat during the task. **C,** Speed vs time data of all the runs carried out in one trial of were aligned to the peak of speed (with averaged speed in dark blue line; see Supplementary Figure 1 for other alignments). Over-trained animals demonstrated a stereotyped kinematic behavior of the runs across the recording session. **D,** Representative example of 3 approach runs illustrates how stereotyped the changes in speed are. The dashed lines represents the locomotion onset and end threshold (upper and lower line, respectively). **E,** Distribution of the durations of all reward approach runs.

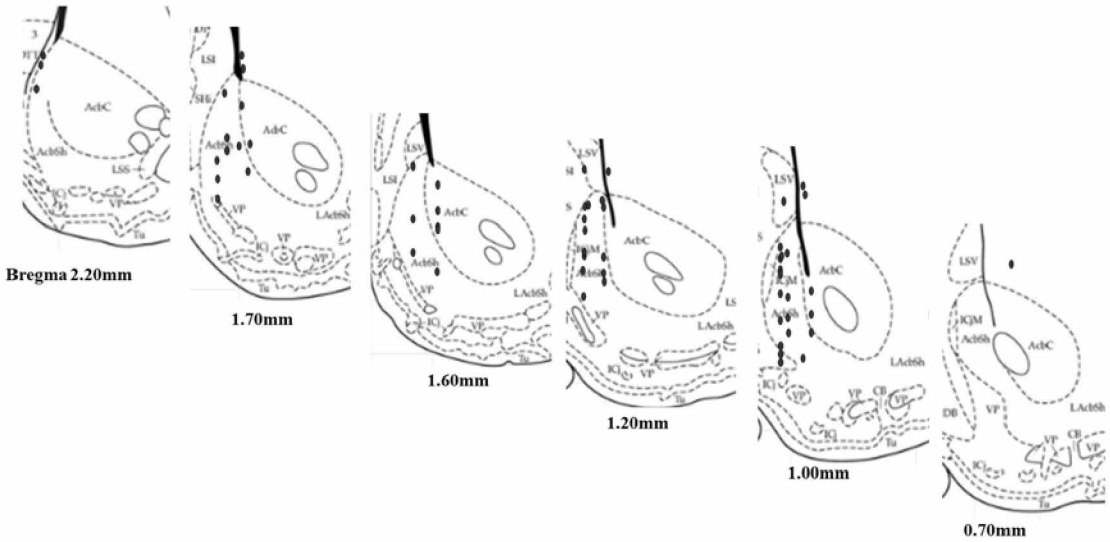


Figure 2: *Putative positioning of the recorded neurons.*

The reconstruction of the recorded neurons position were estimated based on the histology and the distance the electrodes were lowered across the recording sessions. Panels shows coronal atlas sections (Paxinos and Watson, 1998) with the putative positions of recorded neurons in red (dots). Most of the neurons were recorded in NAc shell with a few neurons into other regions as the NAc core, dorsomedial striatum, and lateral septum ventral. Neurons with similar characteristics (as waveforms, mean frequency, and responses) to the ones observed in the NAc shell neurons were kept for later analysis.

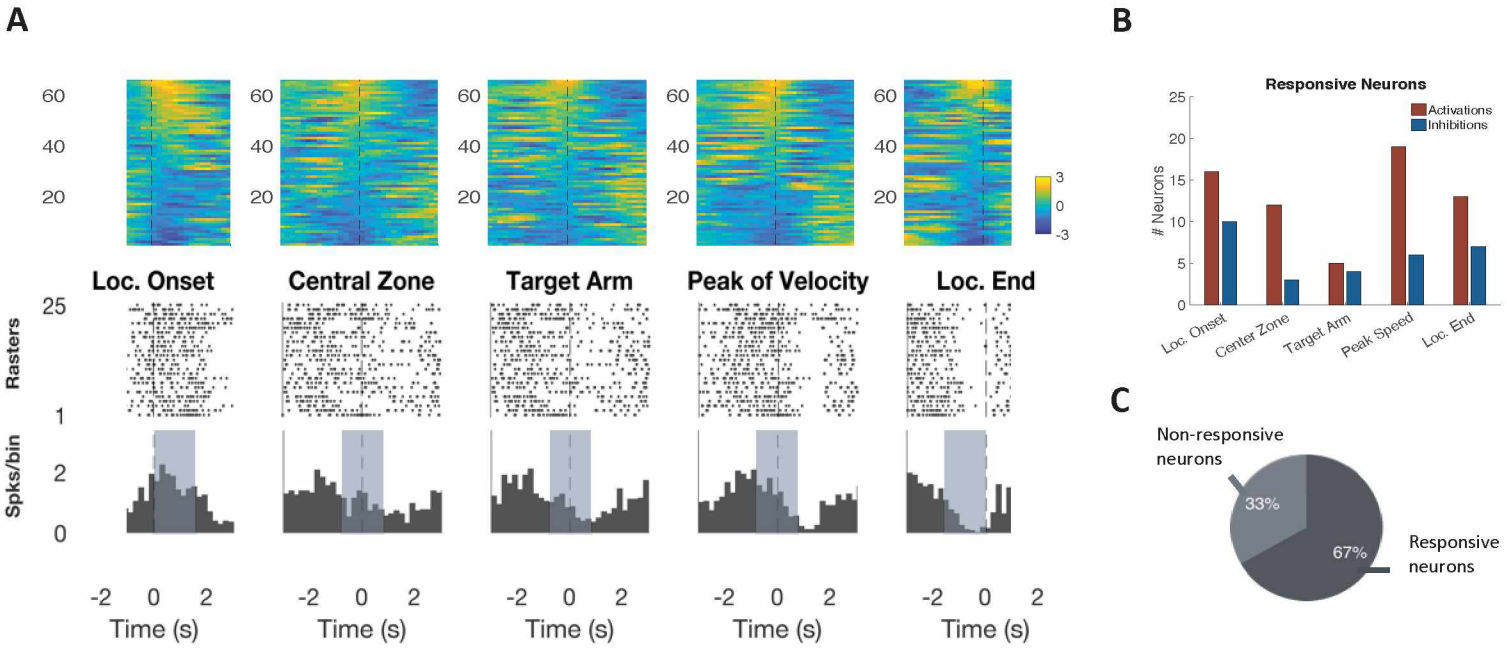
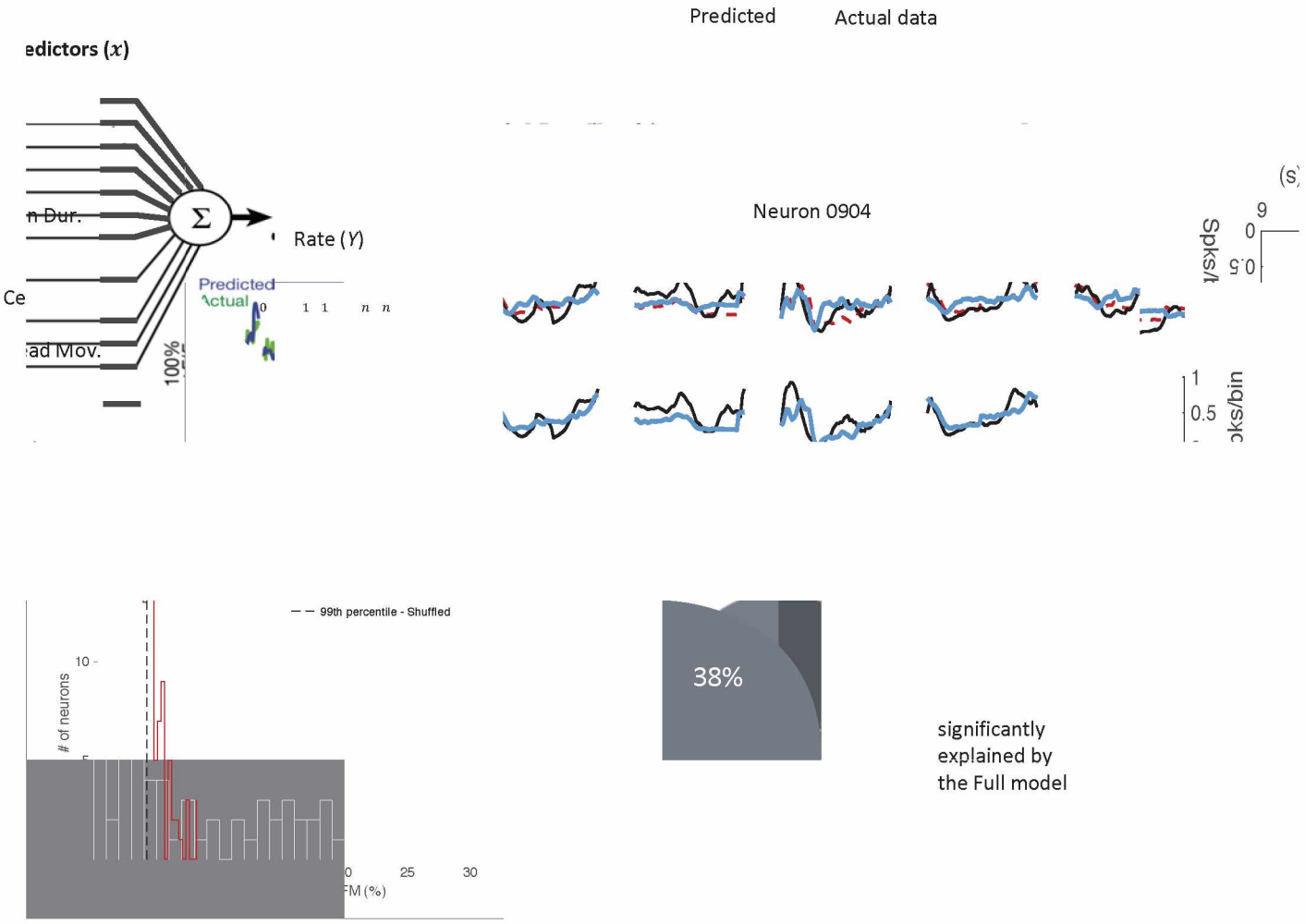


Figure 3: *Responsiveness of NAc neurons to kinematic and behavioral relevant events.*

A, Colored plots show the averaged and z-scored firing rate of all recorded neurons (n = 66) aligned to all five events: locomotion onset, entering in the center zone of the radial maze, entering in the target arm, peak of speed, and locomotion end. The average speed of all approaches of a session is shown below the firing rate histogram. **B,** The number of spikes 0.75 seconds before and after the times the rats entered in the center zone, entered in the target arm, and the peak of speed were counted. Red bars represent the number of neurons with a firing rate higher than the confidence interval firing rate (activation) and the blue bars the number of neurons with a firing rate lower than the confidence interval firing rate (inhibition). Responses to locomotion onset and locomotion end were evaluated 1.5 seconds after or before the events. Shaded areas in the histograms in part **A** represents the windows analyzed for each alignment. **C,** The percentage of neurons that showed significant activation or inhibition to at least one of these events.



re 4: *Generalized Linear Model significantly predicts the neuronal activity of NAc neurons.*

LMs with kinematic and behavioral variables as predictors were used to predict the neuronal activity of the 66 NAc neurons. euronal activity predicted by the GLM compared to the actual data. Five runs from the test datasets are shown. **C,** ibution of the variance explained by the GLMs (encoding model). Red line delimits the distribution of the variance ined by GLM (encoding model) calculated with the neuronal data shuffled (100 shuffled models per neuron). Neurons on ght of the dashed line (99th percentile of the shuffled models distribution) were classified as significantly explained by the l. Black arrows indicates the neurons shown in B. **D,** The percentage of total neurons recorded in NAc that were ficantly explained by the GLM (encoding model).

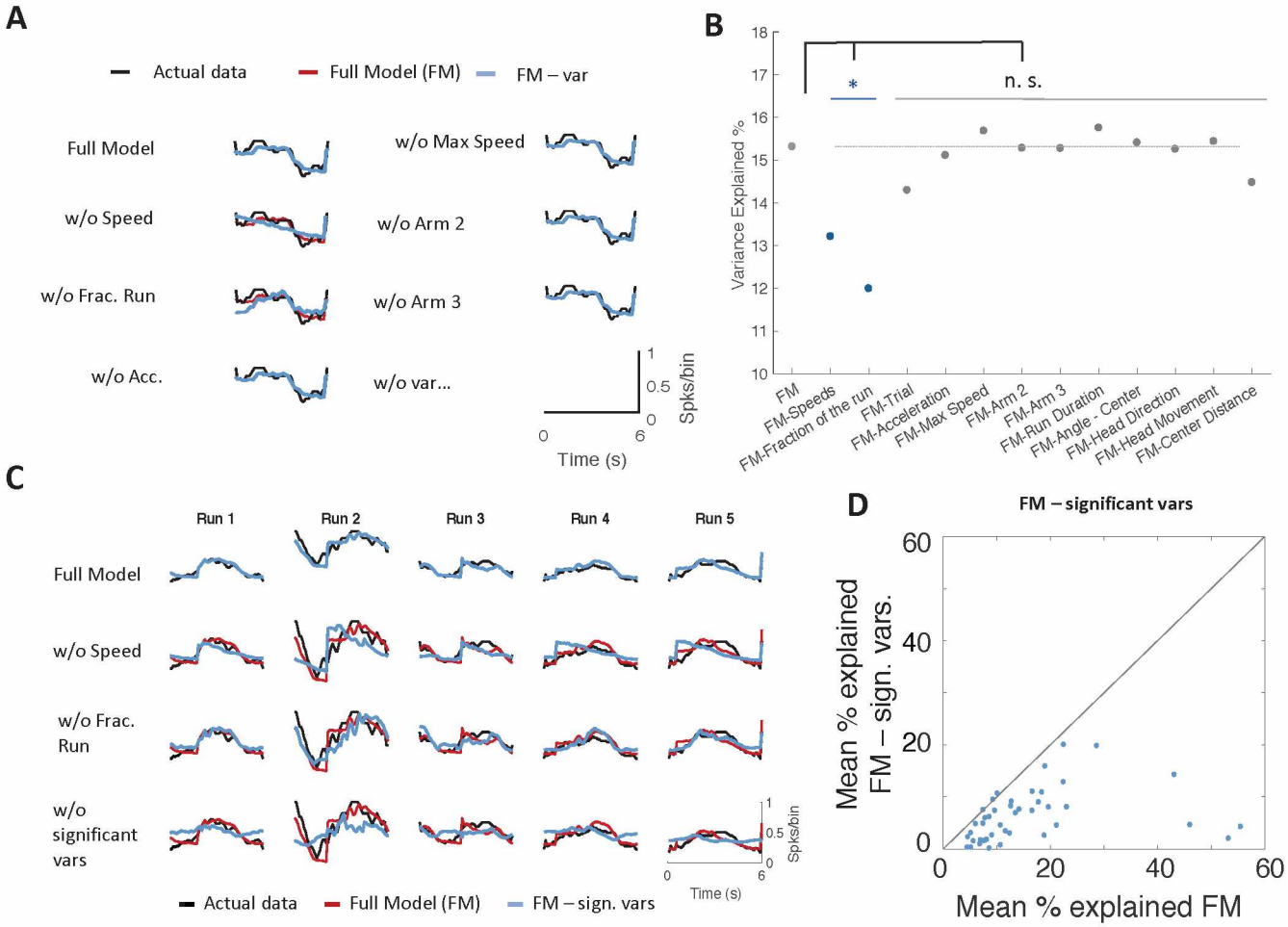


Figure 5: *Speed and fraction of the run represent the predictors with major contribution to the GLM.*

A) To evaluate the contribution of each variable to the GLM, each of the predictors was individually excluded from the model and compared to the full model (FM). Comparison of the actual data (black line), the full model (red line), and the full model excluding each of the predictors (full model-variable) (blue line). **B,** Average contribution (across neurons) of the predictors of the full model-variable compared to the full model (paired t-test after Holm-Sidak correction for multiple comparisons. **C,** Example of a neuron showing the actual data and the predicted data by each model, including one model without both predictors with significant (across neurons) and major contribution to the full model. **D,** Comparison between the full model and the full model excluding the speed and fraction of the run (n=42; the significance of paired t-test after Holm-Sidak method for multiple comparisons: $p=4x10^{-6}$).

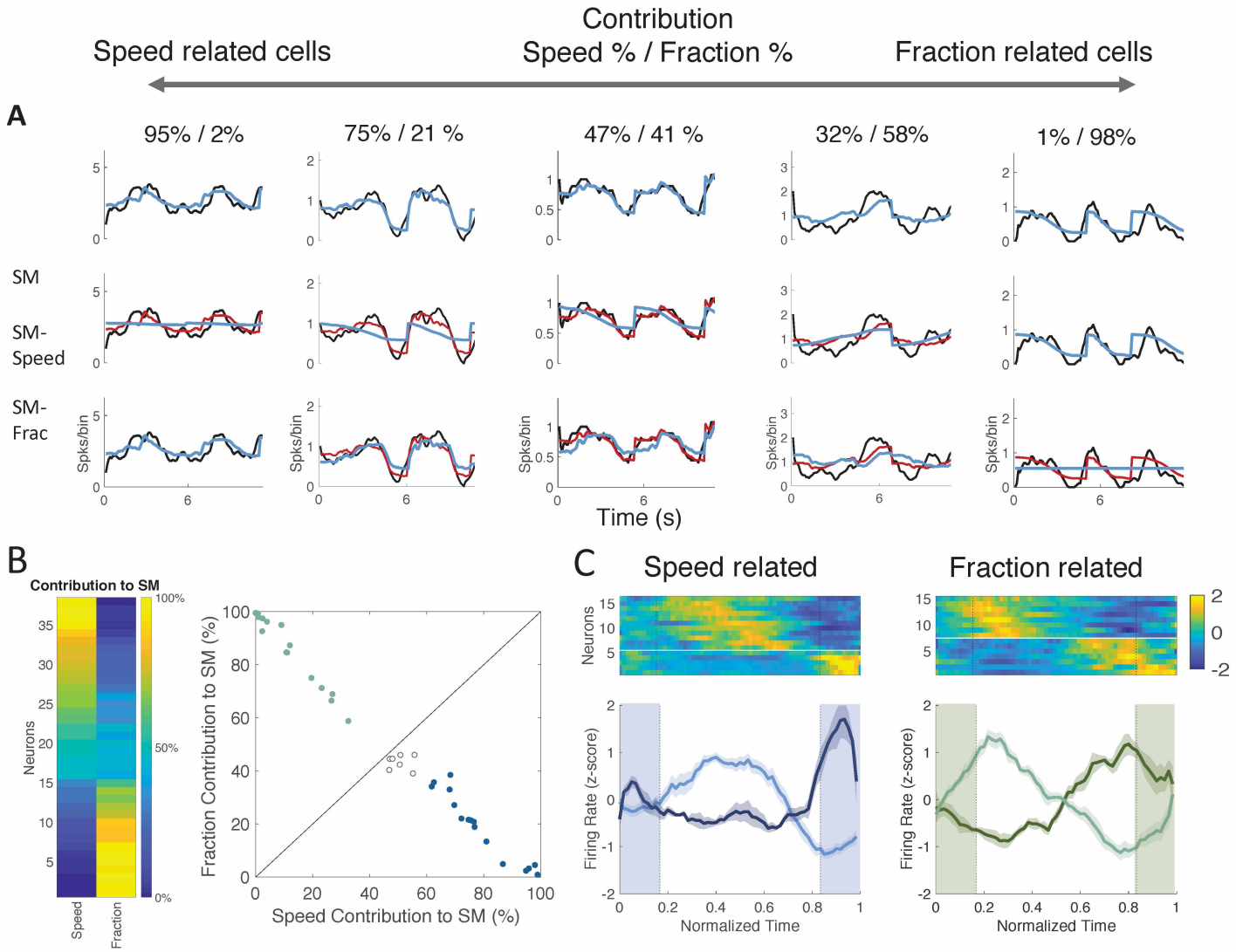


Figure 6: *Speed and fraction related neurons.*

A, Each column represents one neuron with different proportions of the variables' contribution. The first row demonstrate the predicted data by the simpler model over the actual firing rate (18 s data shown; ~1 trial). From left to the right, neurons with major contribution of Speed to the encoding model and neurons with major contribution of fraction of the run variable are shown. The plots show the actual data (black line), the predicted data by the SM (red line), and the simple model less one of the variables (blue line). **B,** A simple model (SM) that use only speed and fraction of the run as predictors was used to calculate the contributions of each of these variables to predict the neuronal activity. The color scaled charts of the 38 neurons that presented a variation in the firing rate significantly explained by the SM are shown. The neurons for which the speed variable contributed 20% more than the fraction of the run to predict the firing rate were classified as speed cells (blue dots). Conversely, the neurons for which the fraction of the run variable contributed 20% more than the speed were classified as fraction cells (green dots). The other neurons are represented by white dots. **C,** Color plot shows the average and z-scored neuronal activity of each speed and fraction related cells (n=13 and n=13, respectively). The two groups were subdivided as positively or negatively correlated with the speed or fraction variable. The white line separates the two subgroups. The four subgroups were named as: +Speed (positively correlated with speed), -Speed (negatively correlated with speed), +Fraction (positively correlated with the fraction of the run), and -Fraction (negatively correlated with the fraction of the run). The averaged activity of each group is plotted below (+Speed represented in light blue line, -Speed in dark blue, +Frac in light green, and -Frac in dark green). Shaded areas define the estimated 1 second before and after the locomotion onset and end, respectively. Shaded error bars represent SEM (standard error).

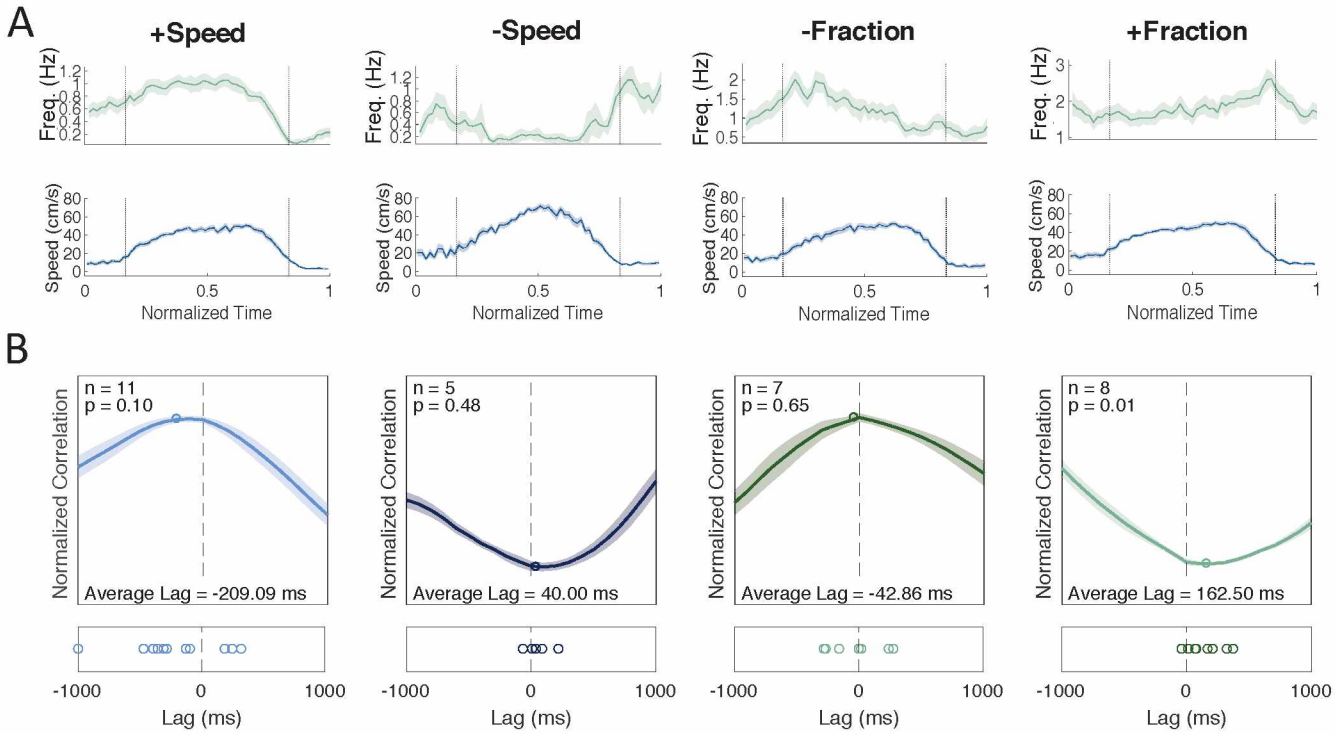
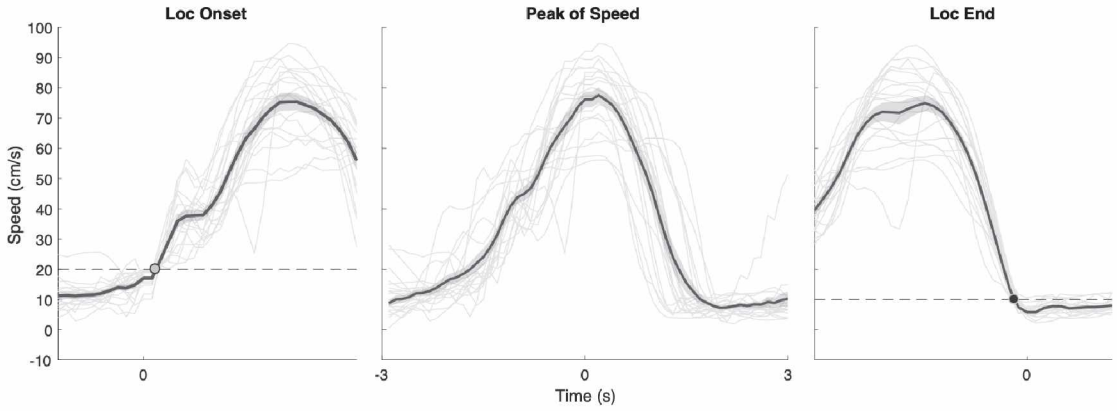
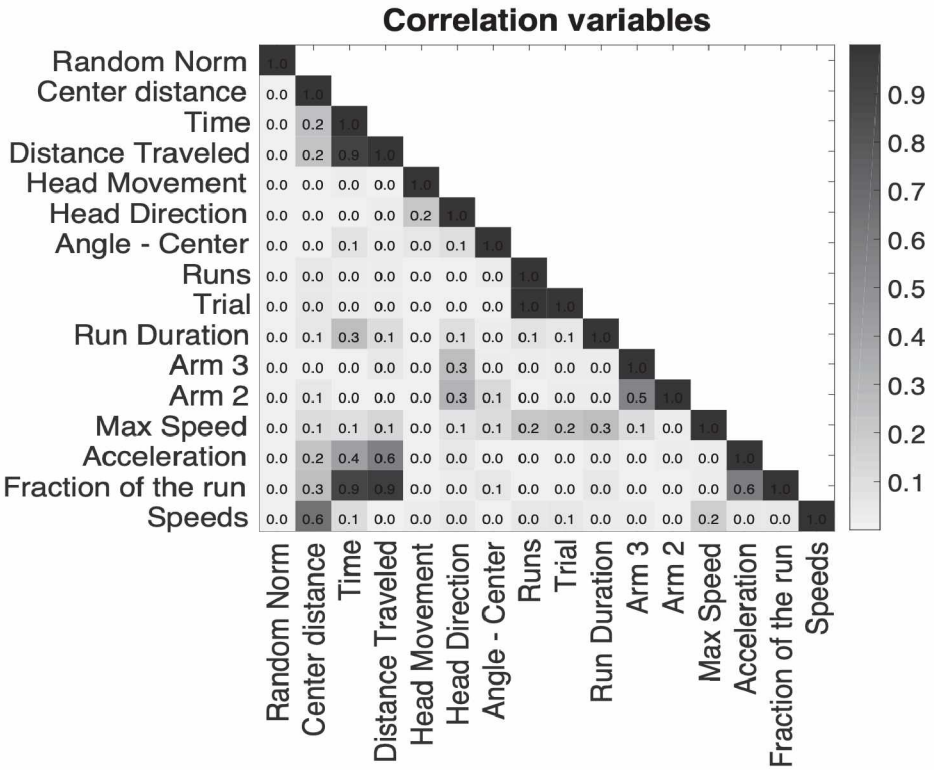


Figure 7: *Changes in firing rate follows changes in speed/fraction of the run.*

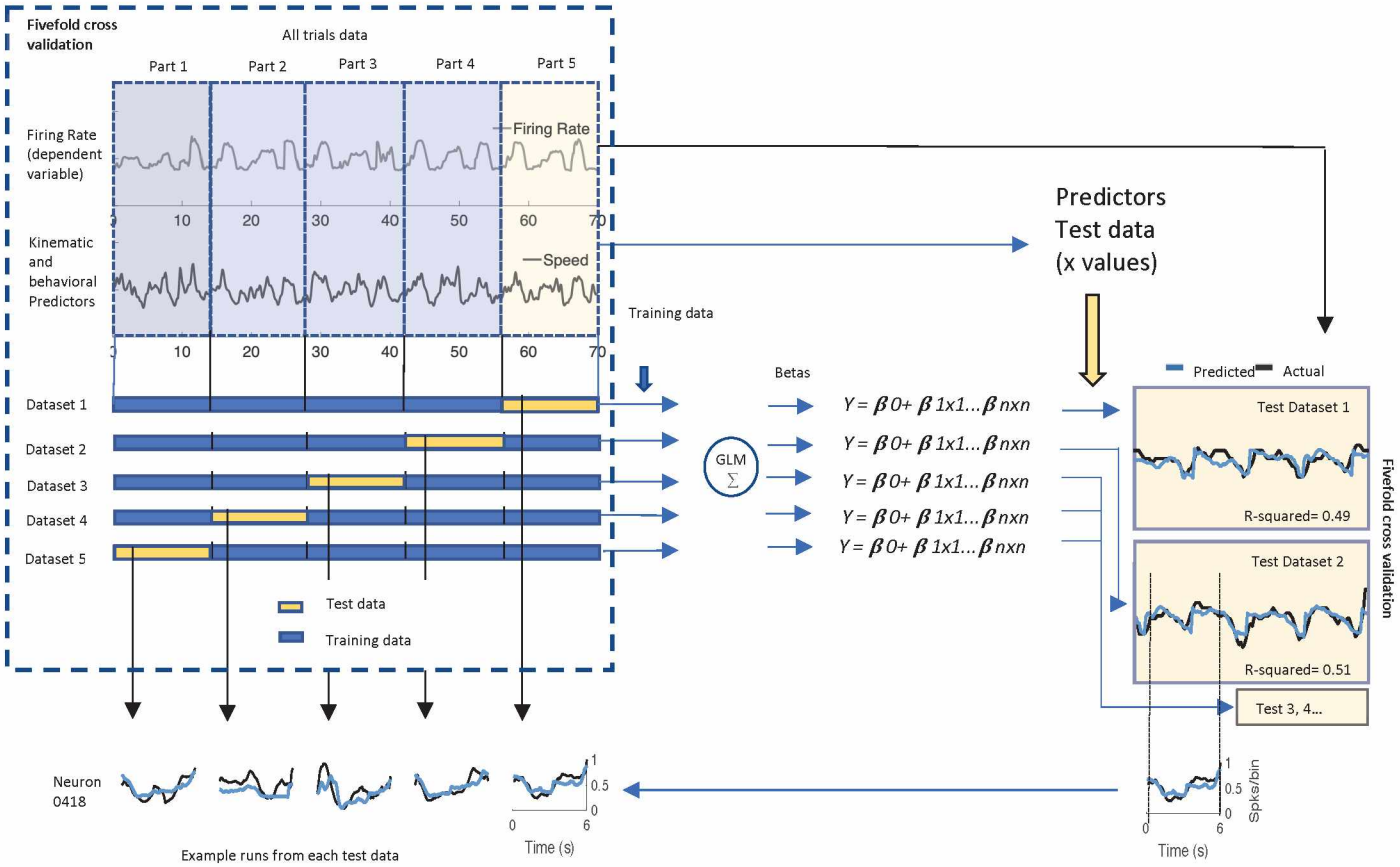
A, The firing rates and speed of all runs were averaged and the time was normalized by the corresponding run fraction. Data came from the same neurons shown in part C (details in Methods). +Speed and -Speed shown in the first column (+Speed and -Speed at the upper and lower graphs, respectively); +Fraction and -Fraction in the second column (+Fraction and -Fraction at the upper and lower graphs, respectively). **B,** A cross-correlation between the firing rate and speed/fraction of the run was calculated to evaluate if the changes in firing rate precedes or succeeds the changes in speed or fraction of the run. The speed/fraction data was time shifted by 100 ms bins forward and backward to find the best linear fit to the variable data and the neuronal data (firing rate). The averaged cross correlation (normalized correlation) for all groups. Blue dot represents the average peak of correlation for all units. Mean correlation maxima ± SEM, one sample t-test: +Speed = -209 ms ±117, p = 0.10; -Speed = +40 ms ± 51, p = 0.48; +Fraction = -42.86 ± 90, p = 0.65; -Fraction = 162.50 ms ± 50, p = 0.01. The "peak" of correlation for individual neurons are shown in the panels below.



Extended Figure 1-1: *Session example of all valid runs (excluded by the efficiency criteria).* Alignments to the locomotion onset, peak of speed and locomotion end. As the approaches runs were stereotyped most of the time, for defining the locomotion onset and locomotion end events, a threshold of speed was used to identify the events. For the locomotion onset, the event was the first time before the peak of speed in which the animal reaches the onset threshold (20 cm/s). The locomotion end was defined as the first time after the peak of speed in which the rat decreased the speed below the end threshold (10 cm/s).

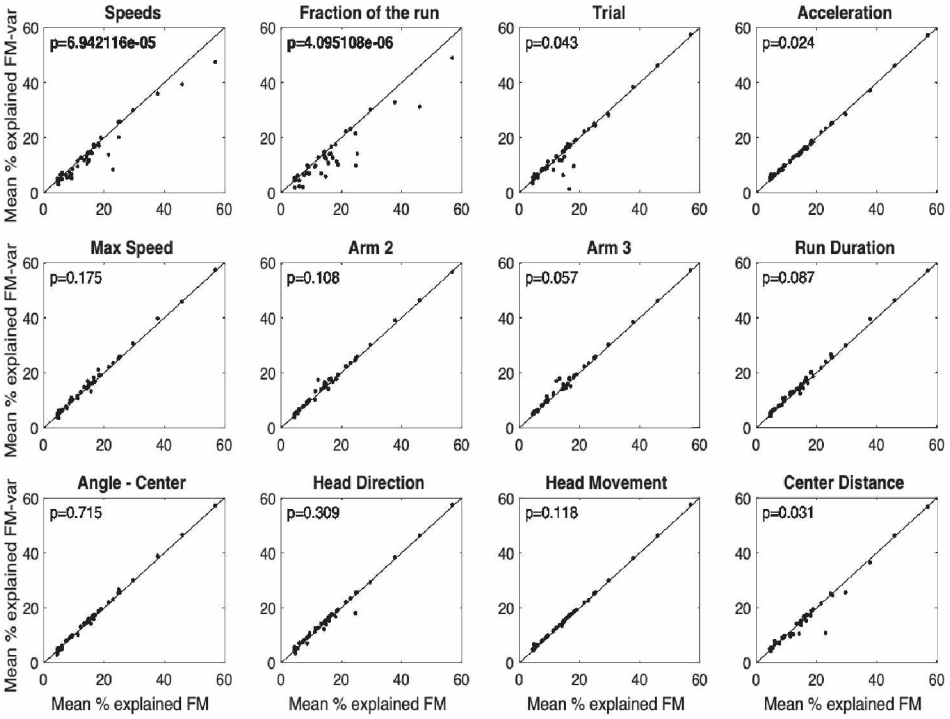


Extended Figure 3-1. *Correlation between variables used in the encoding model.* Behavioral and kinematic variables were selected by the potential contribution to the encoding model. Each variable was correlated with all other variables and, for the pairs with high correlation ($r > 0.8$), one of the pair variables was excluded from the model.



Extended Figure 3-2. *Fivefold cross validation method for evaluating encoding model.*

Firing rate and predictors data of a whole session was divided in 5 parts (5 datasets). Each dataset had the same number of runs (selected randomly for each dataset). 4 of the 5 datasets were selected as training datasets and used to calculate the βs values for each predictor. Then, using the predictors data from test dataset as input to the encoding model (the part of the data not used in training), a predicted data was calculated. The predicted data was compared to the actual firing rate data from the test data set. The variance explained by the GLM was calculated by the R^2 of the correlation between the predicted and actual data. This process was repeated 5 times, the same dataset was not used twice as test data.



Extended Figure 5-1. *Comparison of performance for all neurons explained significantly by the full model.*

Percentage variance explained by the full model (*x* axis) and full model less the one of the predictors (y axis). Each panel shows the comparison between the two models; significance of the paired *t*-test (after Bonferroni correction) is shown in each panel. n=42 in all cases.

3. CONCLUSÃO GERAL

Neste trabalho, por meio de registros eletrofisiológicos de neurônios do NAc de ratos que realizavam uma tarefa de aproximação de recompensas em um labirinto radial de oito braços, nós observamos que a atividade dos neurônios do NAc durante o movimento espontâneo pode ser predito por um modelo que considera variáveis comportamentais e cinéticas. Como esperado, as variáveis mais relevantes do modelo foram velocidade e fração da distância da recompensa (uma forma normalizada de distância da recompensa) (MCGINTY et al., 2013). Algo até então não descrito também foi mostrado pelos nossos dados. Aparentemente, parte dos neurônios do NAc codificam proporções diferentes de informação relacionada a velocidade e fração da corrida. Apesar de termos dividido estes neurônios em *speed* e *fraction related cells* devido a contribuição majoritária das variáveis velocidade ou fração da corrida, foi observado que parte dos neurônios possuem contribuições semelhantes de ambas as variáveis. Isto sugere que as informações de velocidade e distância da recompensa podem ser codificadas de maneira heterogênea pelos neurônios do NAc, de maneira que a codificação de cada variável para cada neurônio seria diferente. Sendo assim, como observado em nossos dados, uma distribuição com um gradiente de contribuições diferentes para cada neurônio, com os neurônios que caracterizamos como *speed* e *fraction related cells* constituindo os extremos da distribuição (neurônios que codificam majoritariamente a informação de apenas uma das variáveis). Nossos dados não apresentaram evidências fortes de que os neurônios registrados durante a tarefa de se aproximar aos três braços recompensados teriam alguma atividade específica para apenas um dos braços. Isto mostra que possivelmente estes neurônios não apresentam relação com a seleção de ações durante a tarefa no labirinto radial.

Em conclusão, este trabalho favorece a hipótese de que os neurônios do NAc codificam informações cinéticas e de proximidade da recompensa durante o comportamento de aproximação espontânea (não condicionado à uma dica) e que envolve a utilização de informações espaciais para se guiar. Embora não tenha sido observado em nosso modelo codificação de seleção de ação (*e.g.* escolha de que braço se aproximar) ou de valor da recompensa, estes resultados não excluem totalmente essa hipótese, pois nosso modelo está limitado a

identificar correlações lineares com os parâmetros avaliados e, ainda, a influência de outros fatores também pode ter sido ignorada. Além disso, a caracterização dos tipos de neurônios (*e.g.* D1 ou D2) que compões os grupos classificados por nós neste trabalho (*+Speed, -Speed, +Fraction* e *-Fraction cells*) seria necessária para esclarecer como estes neurônios integrariam as vias diretas e indiretas dos núcleos da base. Portanto, é proposto a realização de mais experimentos e análises que possibilitariam a diferenciação dos tipos de neurônios que compõe os grupos aqui classificados (*e.g. optotagging*) e a melhor avaliação de outros fatores que podem estar sendo codificados pelo neurônios do NAc.

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