

A SPATIOTEMPORAL EXAMINATION OF THE
MECHANISMS INVOLVED IN LONG-TERM
MEMORY FOR INCREMENTALLY ACQUIRED
INFORMATION

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by
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The existing literature examining the activity of plasticity-related molecular mechanisms suggests that early, post-learning activity of these mechanisms plays a role in later long-term memory (LTM) behavior. However, much of what we know comes from studies using one-trial learning paradigms. This dissertation has two main focuses. First, it examines the role of brain-derived neurotrophic factor (BDNF) in olfactory memories as a means of characterizing the effects of post-learning, molecular mechanisms on LTM for multi-trial learning. Second, it aims to provide a more comprehensive temporal profile for several plasticity-related proteins, across several brain regions for the same multi-trial learning event. In the first study, we showed that blockade of neurotrophin receptors in olfactory bulb (OB), including the BDNF receptor, TrkB, prevents LTM, but not short-term memory (STM) for a multi-trial learning task. In the second study, we found that acute blockade of the BDNF-TrkB pathway did not prevent the formation of specific, short-term odor representations, suggesting that the role of this pathway in the post-learning period is exclusively LTM consolidation. However, its exact role in the specificity of LTM representations remains to be explored. Finally, using high-throughput, quantitative RT-PCR, we explored the timecourse of learning-induced transcription for several plasticity-related proteins across multiple brain regions. This study created a

“spatiotemporal map” of the activity of several molecular mechanisms involved in LTM and lends temporal specificity to future studies of these mechanisms.

BIOGRAPHICAL SKETCH

Tianyi Tong was born on March 18, 1988 in Harbin, Heilongjiang, China. She received the name “Michelle” one fateful afternoon shortly after immigrating to North America in 1994. Divine inspiration struck her parents as they filled out forms of her school while watching an episode of *Full House*. People have been calling her “Michelle” ever since. Michelle graduated from Earl Haig Secondary School (Toronto, ON) in 2006 and received her Bachelors of Science in psychology from Queen’s University (Kingston, ON) in 2010. Her interest in research was piqued during those years through many independent study projects, including projects with Professor Peter Lee (Toronto General Hospital), Professor Nikolaus Troje (Queen’s University), and Professor Martin Egelhaaf (Bielefeld University). After her honours thesis with Professor Li-Jun Ji looking at cultural differences in the use of affect as information in judgments of life satisfaction, Michelle decided that human beings were too troublesome as research subjects. In 2010, she began her doctoral studies with Professor Thomas Cleland at Cornell University where she explored the molecular mechanisms of multi-trial learning in a rodent model.

In August 2015, Michelle married a quiet, engineering type. The two look forward to many years of reading books, avoiding crowded bars, and going to sleep at 21:30.

This dissertation is dedicated to my mom and dad who taught me that
knowledge is nothing without kindness.

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In Chapter 3, the data used to produce Figure 3.1 was collected by Nathalie Mandairon, Francis S. Lee, and Thomas A. Cleland (unpublished data), and is intended for inclusion in a publication based on Chapter 3 of this dissertation. Reproduced with permission.

In Chapter 4, cDNA synthesis and qPCR reactions were performed by research collaborators Madhura Raghavan and Jeffrey A. Pleiss. These data are intended for inclusion in a publication based on Chapter 4 of this dissertation.

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LIST OF ABBREVIATIONS

- AMPA α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ASO antisense oligonucleotides
BDNF brain-derived neurotrophic factor
CaMKII calcium/calmodulin-dependent protein kinase II
cAMP cyclic adenosine monophosphate
CER cerebellum
CREB cAMP response element binding protein
CTX cortex
DMSO dimethyl sulfoxide
E-LTP early long-term potentiation
ERK extracellular signal-related kinase
GABA gamma-Aminobutyric acid
GC granule cells
HPC hippocampus
IA inhibitory avoidance
IEG immediate early genes
L-LTP late long-term potentiation
LTM long-term memory
LTP long-term potentiation
MAPK mitogen-activated protein kinases
NE norepinephrine
NGF nerve growth factor
NMDA N-methyl-D-aspartate
NT-3 neurotrophin 3
OB olfactory bulb
OE olfactory epithelium
OSN olfactory sensory neurons
p75NTR p75 neurotrophin receptor
pCREB phosphorylated cAMP response element binding protein
PGC periglomerular cell
PKA protein kinase A
PKC protein kinase C
PRP plasticity-related proteins
pTrkB phosphorylated tyrosine kinase B
RT-PCR reverse transcription polymerase chain reaction
STM short-term memory
STR striatum
SVZ subventricular zone
TrkA tyrosine kinase A
TrkB tyrosine kinase B
TrkC tyrosine kinase C

CHAPTER 1

PROPERTIES AND MECHANISMS OF OLFACTORY LEARNING AND MEMORY

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1.1 Introduction

Olfactory learning is distributed across multiple regions of the brain. Studies of learning within associative brain regions such as hippocampus (HPC) and pre-frontal cortex – particularly in rodents – can utilize and manipulate olfactory stimuli just as they do other forms of sensory input [55, 56, 152, 108]. Importantly, however, a substantial component of odor learning is intrinsic to the olfactory bulb (OB), to which primary olfactory sensory neurons (OSNs) directly project, and to its interactions with the piriform (olfactory) cortex, to which the mitral cells (second-order sensory neurons of the OB) project. Within OB proper, several lines of evidence, including NMDA-based synaptic plasticity [136, 186], the long-term potentiation of ascending piriform pyramidal projections onto OB granule cells [67] and odor memory persistence linked to the selective retention of adult-born interneurons in the OB [98, 144, 178] indicate that the OB itself supports sophisticated intrinsic plasticity mechanisms that regulate the trans-

formation of olfactory signals across the first principal sensory synapse.

The elucidation of these intrinsic learning mechanisms within OB presents both theoretical and practical opportunities. While the OB is highly interconnected with multiple cortical and subcortical regions, it is relatively morphologically isolated. This facilitates, for example, the specific delivery of neurochemicals or virally-packaged transgenes to the OB via cannulation. The neural circuitry of the OB and the physiology of its diverse neurons are reasonably well-described, enabling the development of biophysically realistic models of OB function that can associate specific cellular properties and mechanisms with systems-level function and performance [113, 138]. Specific odor-dependent behavioral paradigms have been developed that are strongly sensitive to OB manipulations and are likely to depend on OB intrinsic learning, enabling some segregation of OB-specific learning from odor learning dependent on other brain regions [188].

As an introduction to my empirical work, I discuss how research into cumulative, appetitive, representational learning mediated by plasticity in the early olfactory system can productively contribute to a broad understanding of general learning and memory mechanisms. I also provide a comprehensive review of the existing work on the structural and molecular mechanisms of memory formation, with a focus on the timecourses of learning-initiated signaling cascades and the roles of extracellular signals such as classical neuromodulators and the peptide brain-derived neurotrophic factor (BDNF).

1.2 Learning in the olfactory system

1.2.1 Appetitive odor learning is cumulative and incremental

The cellular and synaptic neurophysiology of mammalian learning and memory is substantially based on fear conditioning. The advantage of the conditioned fear model is that strong, discrete, and easily measurable memories can be generated by single learning trials, avoiding the complexity and additional questions imposed by the need to integrate the cumulative effects of multiple learning events. The persistence of these memories is a function of the unconditioned stimulus amplitude – e.g., footshock current – but commonly extends to several days [15], enabling study of the sequential transitions in their structural, biochemical, and molecular substrates that occur over time. The clearest example of these gradually transforming dependencies is the protein synthesis requirement for long-term (many hours to days or more) but not short-term (up to a few hours) memory [48, 53], though additional phases of memory have been defined in some systems. Moreover, many specific cellular signaling cascades, induced by fear conditioning events and underlying the relevant learning, have been described; whereas most of these processes are initiated immediately after the causal event, several have been described that are initiated minutes or even hours later (Fig. 1.2).

Olfactory appetitive learning, in contrast, is gradual, cumulative, and statistical. The richness of the OB learning model that is gained by its statistical and representational character also imposes a cost in terms of unavoidable complexity. For example, the distribution of repeated training events in time will always be a factor; *massed* versus *spaced* learning schedules are well known to

affect memory persistence [137, 184, 98], and intertrial interval timing can even determine which areas of the brain are most immediately responsible for nonassociative odor learning [136]. This complexity, however, is manageable, and is substantially mitigated by the theoretical tractability and experimental accessibility of the OB as well as the previous elucidation of plasticity-related molecular cascades in other cortical memory systems. In Chapter 2, we describe an experiment using a olfactory learning paradigm to investigate the differential involvement of the BDNF-TrkB pathways in STM and LTM for incrementally acquired information.

1.2.2 Odor learning is representational

Learning alters the transformation of information by a neural circuit, and *memory* refers to the persistence of that altered transformation function over time. In olfactory representational learning, the forms of odor representations are sensitive to learning and can be measured using behavioral generalization gradients [41, 42, 59]. Olfactory generalization gradients define the range of variance in odor quality that an animal will respond to as representative of a given odor, and reflect the statistical reliability of odor features [193]. The area under the gradient, or *consequential region* [172], describes the degree of certainty expressed by the animal that a stimulus of a given quality is likely to represent that learned odor or its implications. The *forms* of generalization gradients are strongly influenced by learning. Increased pairings of odor with reward progressively narrow and sharpen the generalization gradient, and manipulations of other training parameters indicate that factors that increase classical learning also increase the rate of sharpening of olfactory generalization gradients [42]. If the odor being

paired with reward is itself variable in quality, however, it becomes clear that the generalization gradient does not sharpen *per se*, but progressively conforms to the actual environmental distribution of reward-predicting odor qualities as experienced by the animal [40]. That is, the learning-dependent regulation of generalization gradients describes a statistical learning process by which an animal’s internal odor representations become gradually and probabilistically categorical, evolving to correctly reflect the meaningful categories of the external olfactory world. This aspect of odor learning has been hypothesized to rely on OB circuitry both for theoretical reasons and based on results from the experimental manipulation of OB circuit function [49, 51, 54, 73, 115, 124, 136]. Hence, in contrast to *odorants* – which are chemical stimuli, whether simple or complex – *odors* here are psychometrically defined as probability density functions of odorant quality that the animal has learned imply the same consequences, embedded within a high-dimensional similarity space that is best defined by odorant receptor activation levels [38, 39]. Behaviorally-measured generalization gradients constitute one-dimensional trajectories within this high-dimensional space. In Chapter 3, we use these “generalization gradients” to investigate the role of the BDNF-TrkB pathways in representational learning.

1.2.3 Behavioral methods and advantages of olfactory learning models

The OB provides both practical and theoretical advantages for study of the molecular and structural mechanisms involved in memory. Practically, pharmacological agents can be infused selectively and locally into the OB. Intrinsic

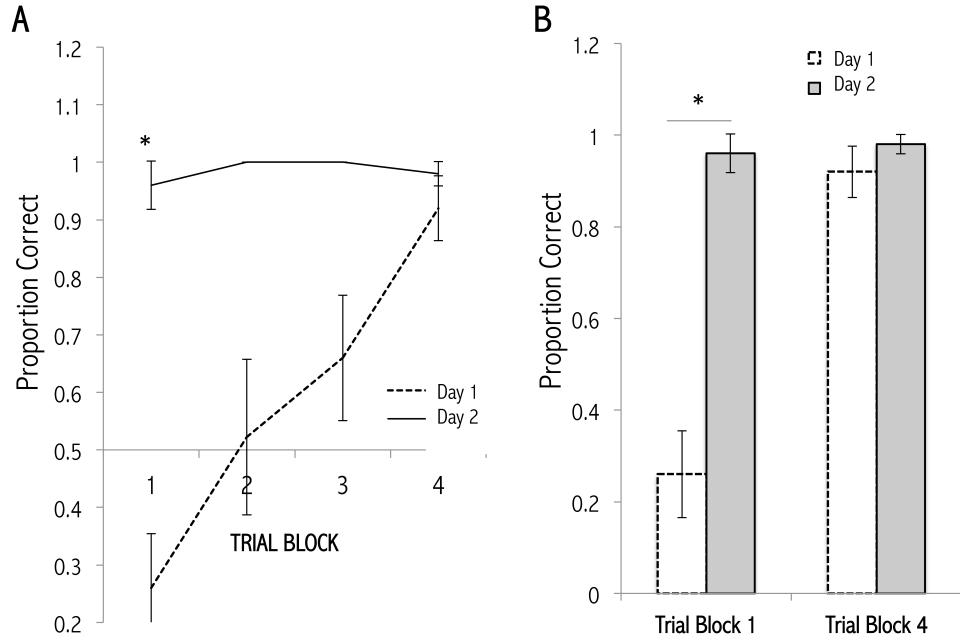


Figure 1.1: **(A)** Mice received 20 trials of discrimination training in which they learned to choose a rewarded conditioned odor (1.0 Pa) over a distractor odor (Day 1). Twenty-four hours later, the discrimination training was repeated (Day 2). The correct trials were scored and averaged across animals. Trials are grouped into 4 blocks of 5 consecutive trials for display and analysis. A steady improvement across trials on Day 1 is remembered one day later. **(B)** Comparing trial block 1 (trials 1-5) between days, mice performed significantly better on the second training session, indicating a robust retention of odor memory ($t(8) = 7.5593, p < 0.001$). Figure adapted from [183].

OB circuits display functional plasticity similar to other regions of the brain, including LTP [67] and adult neurogenesis [117], and are reconfigured substantially by neuromodulatory inputs [51]. Established behavioral paradigms enable insight into the changing form as well as the persistence of odor representations over time, and physiological studies enable measurements of direct correspondence between environmental changes, behavioral performance, and the synaptic and molecular changes that occur in neural circuitry [2, 1, 158]. In particular, odor learning exhibits varying memory durations that are related to

behavioral task parameters and depend on evolving physiological substrates for short-term memory [136], intermediate-term memory [72], and long-term memory (Fig. 1.1) [109, 125].

Habituation and spontaneous discrimination In this non-associative olfactory learning paradigm, animals first are habituated to an odorant, responding to repeated presentations with progressively lower investigation times. Some time after habituation, they are presented again with that odorant, or with a series of structurally and perceptually similar odorants. Perceptually distinct odors elicit normal, non-habituated investigation times, but odorants similar to the habituated odorant elicit reduced, partially-habituated responses depending on the degree of similarity between the habituated and test odorants. Importantly, the non-associative nature of this paradigm means that increases in investigation times to differing stimuli are spontaneous and do not result from experiment-specific associations (*spontaneous discrimination*). Interestingly, memory for odorant habituation acquired on short timescales (tens of seconds) is predominantly mediated within piriform cortex [187], whereas habituation on the minutes timescale is localized within OB [34, 136]. The persistence of habituation and spontaneous discrimination is sensitive to the degree of habituation, declining over a 10-20 minute period in standard protocols [62]. Both the extent and persistence of discrimination memory are regulated by neuromodulatory and hormonal effects in the OB as well as task parameters and state variables [54, 124, 126, 127].

Associative generalization. “Generalization gradients” can be measured in response to odorants that are conditioned via associative pairing with reward

[41, 42]. After conditioning, animals are tested with batteries of structurally and perceptually similar odorants, often in a digging task where the odorant cue signals a buried reward. The animals' perseverance in pursuit of an expected reward (that is not present in test trials) declines with increasing perceptual dissimilarity between the conditioned and test odorants. The breadths and forms of these gradients are sensitive to determinants of learning and to the statistical variance in odorant conditioned stimulus quality across conditioning trials [42, 40] and also are sensitive to the pharmacological and neuromodulatory manipulation of OB circuitry [44, 43, 199]. Associative odor learning based on a standard short-term conditioning paradigm (a single series of up to twelve massed conditioning trials) progressively decays over a timescale of several hours, though this timescale is likely to be sensitive to training parameters.

Odor discrimination. Odor discrimination is the most commonly used olfactory learning model, and subsumes many radically different conditioning paradigms and performance metrics. The distinguishing feature of this task is that animals are motivated to distinguish between two or more odors with different learned contingencies (e.g., one is rewarded and the other not), such that it tends to measure an animal's capacity to learn a given discrimination rather than to measure an odor representation *per se*. Automated tasks with relatively nonintuitive metrics (e.g., odor-specified left-right selection or go/no-go tasks) may utilize hundreds of training trials, whereas tasks with more intuitive (to the animal) metrics such as odor-cued digging often requires no more than 20 trials to reach criterion after the task is learned. The dependence of odor discrimination performance on OB circuitry corresponds closely with the difficulty of the discrimination, which corroborates theoretical proposals that OB circuitry

serves in large part to identify which statistical differences among inputs correspond to meaningfully different odorants, and which are simply variations of a single odor that should be generalized [40]

Olfactory performance depends on memory. In olfaction, memory does not serve only to remember odors past, but is also a critical factor in realtime perceptual processing even within OB and piriform cortex [189, 190, 200]. Hence, short-term and long-term memory processes are likely to be highly interactive and conditional; e.g., the form of a long-term memory should acquire the evolving characteristics of accumulating short-term memory processes during multi-trial odor learning tasks or natural learning scenarios. That is, though it is established in general that STM and LTM processes are initiated separately – i.e., LTM is not simply a continuation of STM (Fig. 1.2) [82] – it is also true that LTM must be able to be repeatedly updated based on new information even before it is first behaviorally expressed. One likely scenario is that short-term learning and memory processes contribute to this updating – a hypothesis that the olfactory appetitive learning and memory model is well-poised to test.

1.3 Molecular and structural mechanisms of learning and memory, in general

Memory mechanisms are heterogeneous in form, structure, and timecourse, yet exhibit many commonalities across regions of the brain. Presently, the neural mechanisms are discussed in two broad categories: *molecular*, which includes intracellular cascades, molecular signaling, neuromodulatory influences, activity-

dependent protein synthesis, and epigenetic modifications thereof, and *structural*, which includes physiological changes such as long-term potentiation or other synaptic weight changes, alterations to neuronal morphology such as dendritic branching, changes to terminal shapes or numbers, and ancillary modifications such as effects on glia or cell adhesion to the extracellular matrix, as well as, changes to neuron number via adult neurogenesis or selective apoptosis. Mechanisms from these categories often are interdependent, and exhibit characteristic response timecourses that underlie memory-related changes. We review established mechanisms of plasticity in non-olfactory and olfactory circuits, with a focus on their timecourses and their implications for how memories are constructed. We provide a brief review of the more researched mechanism here as a means of establishing knowledge to discuss parallel mechanisms for research in OB-dependent learning.

1.3.1 Molecular mechanisms

Inhibitory avoidance (IA) is a well-established behavioral paradigm for one-trial fear conditioning that offers a simple analogue measure of memory “strength” and can persist strongly for days, enabling study of both short-term and long-term memory mechanisms. If entering a darkened chamber or stepping down from a platform results in footshock on the conditioning trial, a normal animal will hesitate, in subsequent test trials, before again entering that chamber or stepping down. The delay in seconds before again entering the chamber or stepping down is a robust measure of the strength of the action-consequence association. Much of what is known about the molecular mechanisms of memory and their timecourses in mammalian systems has been developed using this

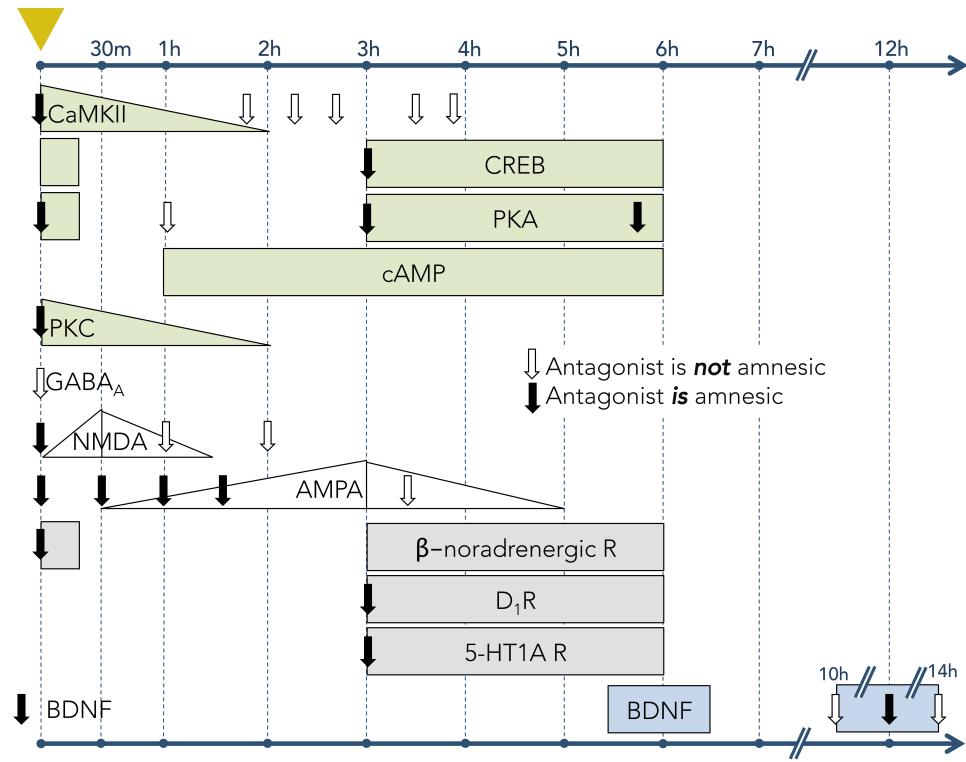


Figure 1.2: Timecourses of activity for learning-associated molecular mechanisms in hippocampal tissue following one-trial inhibitory avoidance (IA) training. Leftmost dashed vertical line (marked with yellow triangle) denotes the time of IA conditioning. Subsequent vertical lines denote timepoints following the trial. Rows correspond to particular mechanisms (signaling molecule, receptor, or neuromodulator). Downward-pointing arrows indicate timepoints when antagonists to the corresponding receptor or signaling pathway were infused into the HPC; solid arrows denote that the antagonists had an amnesic effect on LTM (when tested 24-48 hours after conditioning). Figure adapted from [183].

task.

Long-term memory. IA conditioning leads to a rapid elevation in calcium/calmodulin-dependent protein kinase II (CaMKII) levels in the HPC. This in turn enhances the phosphorylation of cyclic AMP (cAMP) response element binding protein

(CREB) [140] and promotes the formation of complexes with ionotropic glutamate NMDA receptors[168]; these receptors have been shown to play a functional role in learning and memory (reviewed in [47]). Blocking CaMKII activity immediately after IA training substantially reduced animals' fear responses when measured 24 after initial training (i.e., LTM). However, blocking CaMKII activity 30 minutes after IA resulted in a weaker LTM deficit, and blockade 2-4 hours after IA had no effect on LTM at all [192] (Fig. 1.2). Further studies corroborated these findings by directly measuring CaMKII levels, revealing that CaMKII activity increased immediately after IA training, and remained high when tested 30 minutes later, but had returned to baseline when tested two hours after conditioning [20]. These findings indicate that CaMKII plays a crucial role early in the memory induction process, and that its functional role in LTM formation is confined to a specific period following learning. Particularly relevant to this dissertation, the neurotrophin, BDNF, also plays a critical signaling role in LTM induction (Fig. 1.2). For example, blockade of BDNF signaling through its TrkB receptor disrupted LTM but not STM for a conditioned IA event, whereas infusion of recombinant BDNF into HPC rescued IA memory from amnesia induced by glucocorticoid receptor blockade [35].

Other studies have demonstrated the early involvement of the cAMP-protein kinase A (PKA)-CREB pathway in LTM formation. Cyclic AMP levels in the HPC begin to rise about 30 minutes following IA training, peak at three hours after training, and decrease to baseline levels circa six hours after training [22, 20]. PKA activity and phosphorylated CREB (pCREB) levels, in contrast, exhibit two distinct peaks: one immediately following training and another beginning three hours after training; this second wave coincides with peak hippocampal cAMP levels and remains elevated 6 hours after training [20]. Injec-

tions of PKA antagonists into the HPC at the onset of the second PKA wave have amnesic effects on IA memory when tested 24 hours after training [20]. Similarly, injections of antisense CREB into the amygdala impaired LTM in the IA task [32], and hippocampal infusions of antisense CREB oligonucleotides prior to water maze training blocked 48-hour LTM while sparing 4-hour STM [74]. CREB mutant mice also exhibit impaired LTM consolidation, but normal STM, on a contextual fear conditioning task [29]. PKA/CREB activity during the second peak period after IA training (3-6 hours following IA) is also followed by synthesis of the immediate-early gene, *c-fos* [80], a transcription factor often expressed before biochemical events associated with learning [155]. Following one-trial olfactory appetitive conditioning in neonatal rats, PKA activity in the OB increased after 10 minutes. Blocking this PKA activation with Rp-cAMPs, specifically at 10 minutes, blocked subsequent memory for the conditioned odor when tested 5 hours or 24 hours, but not 3 hours, after training. Moreover, exogenous administration of PKA into the OB 10 minutes after conditioning extended the duration of a normal 24-hour memory to 72 hours [71].

Studies of these mechanisms in multi-trial learning has been spare. Appetitive learning paradigms, such as a radial arm maze task, show no increase in PKA activity and CREB phosphorylation immediately after the first training day, but find, instead, detectable increases immediately after the fourth day of training [142]. This finding suggests that in multi-trial learning paradigms these pathways have a more complicated activity profile that includes variations on the order of days, as well as minutes. A similar pattern has been described with learning-associated BDNF activation. IA training results in immediate increases in BDNF levels in the HPC. Infusions of function-blocking BDNF antisense oligonucleotides into the HPC prior to, but not 6 hours after, IA training

block LTM, indicating that BDNF activity immediately following learning sets the stage for eventual LTM consolidation [7]. Similarly, BDNF mRNA levels in HPC increased significantly 15 minutes (not immediately) after 8, but not 4, days of conditioning on an appetitive radial arm maze task [142] and this 15-minute increase in BDNF mRNA levels is still seen after the 28th day of training [143]. It is unclear whether the levels at 8 and 28 days represents a continuous elevation in BDNF mRNA across those days or a reflection of multiple peaks in BDNF mRNA activity. These findings, however, do show that similar molecular mechanisms are involved in multi-trial appetitive learning as in fear-based single-trial learning, but that the timecourse and other contingencies of learning differ. It is in these contingencies that the richness of appetitive learning studies is likely to contribute most significantly to general studies of learning and memory. Should LTM be modeled as an evidence accumulation system, where LTM is formed only after enough evidence has accumulated that the cue-reward association is reliable and likely to remain true over time? How is this compatible with the evidence that, in IA training, LTM induction is initiated immediately after learning (and is not dependent upon intact STM), even though it cannot govern behavioral responses until hours later? In Chapter 4, we broaden our investigation beyond the BDNF-TrkB pathway in the OB and examine the time-course of several memory-related mechanisms (including those described here) following multi-trial learning.

1.3.2 Structural mechanisms

The examination of molecular mechanisms are important because many of the mechanisms characterized by behavioral studies to be involved in LTM induc-

tion and maintenance have also been shown to influence persistent structural changes to neuronal ensembles. These changes may in some cases be the primary effectors of the memory. We briefly review some of these structural mechanisms here.

Long-term potentiation. Over decades of research, considerable debate has arisen about whether, and to what extent, long-term synaptic potentiation (LTP, [27]) underlies or otherwise corresponds to behaviorally-measured LTM [79]. The arguments in favor of their relationship were strengthened by the elucidation of two distinct forms of hippocampal long-term plasticity (LTP), a short-duration early form (E-LTP) and a longer-lasting late form (L-LTP) distinguished primarily by the latter's dependence on protein synthesis. Specifically, the persistence of LTP in the CA1 region beyond roughly four hours depends on mRNA and protein synthesis [26, 65]; translation blockers injected into rat dentate gyrus during *in vivo* LTP induction caused synaptic potentiation to decay within 3-4 hours [104]. This timescale closely resembles the protein-synthesis dependency of LTM observed in behavioral studies. Similarly, after LTP induction by a tetanic stimulation of afferent fibers in hippocampal slices, any further tetanus to the afferent within three hours generates only short-term plasticity across the synapse, whereas after four hours, in contrast, the same tetanus could generate a longer-lasting potentiation over and above the initially induced LTP level [64]. The timescale of this effect also corresponds with the STM/LTM distinctions described above, and additionally suggests that LTM expression may free up resources needed for further learning.

Finally, several molecular mechanisms associated with memory induction and persistence also regulate LTP. For example, CaMKII activity within the first

five minutes following LTP induction is crucial for the maintenance of synaptic potentiation [162], PKC inhibition immediately following induction leads to early decay of potentiation [77], and inhibitors of PKA limit the persistence of LTP to roughly three hours [63]. BDNF also facilitates the induction of LTP in hippocampal slices [103], and application of BDNF in the presence of protein synthesis inhibitors is sufficient to transform a short-lasting LTP to a longer-lasting form [119], suggesting a role for BDNF in the determination of long-term functional plasticity that is comparable to its necessary and sufficient role in determining LTM persistence [14]. Moreover, blockade of BDNF signaling immediately following LTP induction reduced LTP persistence. Specifically, LTP induction in slices generated a transient peak in the phosphorylated form of the TrkB receptor (pTrkB) for BDNF; pTrkB levels rose 15 minutes following induction, peaked at 30 minutes, and slowly declined to baseline over two hours [120]. Blockade of TrkB receptors at the 30-minute peak, but not at 60 minutes post-induction, inhibited persistent LTP [93]. The timecourses of these interactions also correspond to those of the early mechanism involved in LTM formation as previously discussed.

Neuronal and synaptic morphology. Changes in neuronal morphology, such as the growth of new dendritic spines, have been shown to accompany novel experiences [90, 110]. Importantly, stabilized new dendritic spines underlie at least some LTMs [196], indicating that durable modifications of the synaptic weights within neuronal networks mediated by physical spines is a structural mechanism underlying memory persistence (reviewed in [159, 173]). The specific roles of these morphological elements are further emphasized by the dependence of long-term memory on intact cytoskeletal dynamics [107]. Notably,

BDNF and other neurotrophins associated with memory regulation have been strongly implicated in the modification and maintenance of both synaptic efficacy and dendritic morphology (reviewed in [33, 132, 197]).

Adult neurogenesis. The proliferation of new neurons ceases prior to adulthood in most brain regions, with the exception of the HPC and OB, and possibly the hypothalamus [37]. Hippocampal progenitor cells are produced in the subgranular zone of the HPC and migrate a short distance to the granule cell layer of the dentate gyrus; in contrast, OB progenitor cells are produced in the subventricular zone (SVZ) and migrate to the OB along the rostral migratory stream for 10-14 days before arriving in the OB and differentiating within the granule cell and glomerular layers [153]. The observation that olfactory learning increases the odor-specific survival of adult-born neurons in OB [98, 178, 8] and, conversely, that the selective activation of these adult-born neurons facilitates olfactory performance and memory [6] has led to a broad and well-supported hypothesis that adult neurogenesis underlies LTM in OB as it does in the HPC (reviewed in [69, 111, 166]). However, the observation that this constant integration of new neurons does not result in a progressively increasing total neuron number in the OB [147] suggests that these new neurons may be relatively short-lived, or may replace older neurons, or both, rendering unclear some essential aspects of the role of adult neurogenesis in long-term odor memory within OB.

In the hippocampus, environmental enrichment and experience increase the survival rates of adult-generated neurons within the dentate gyrus [96, 180]. Moreover, critically, the selective destruction of adult-born neurons that recently had been incorporated into the hippocampal network impaired spatial memory in the Morris water maze task when animals were tested either days after train-

ing [11]. This latter result indicates that these newly-incorporated neurons were substantially mediating the new spatial memory; indeed, it has been suggested that adult-born neurons in HPC are employed specifically for new learning (i.e., initial acquisition), as opposed to the expression or reacquisition of memory [9]. A similar principle is emerging in the OB, within which the selective ablation of newly-incorporated adult-born neurons following appetitive odor conditioning eliminated animals' memory for that odor [4].

Interestingly, some of the signaling mechanisms most strongly associated with LTM formation also appear to be involved in the learning-dependent survival of adult-born neurons. Besides a basic activity-dependence arising from glutamate and GABA receptor activation [157, 156], the survival of adult-born neurons is also enhanced by stimulation with NE [145, 185] or BDNF [169]. For example, infusions of BDNF into the hippocampus, when delivered to adult rats over two weeks, increased the number of adult-born granule cells when compared against control animals infused with saline vehicle or bovine serum albumin [169]. In heterozygous BDNF knockout mice, the number of surviving new neurons in the hippocampus did not change (despite increased proliferation in the subgranular zone); however, adult-born neurons continued to express markers of immature neurons as well as reduced dendritic growth, suggesting that reduced BDNF levels impaired their processes of maturation and differentiation. Other studies have emphasized a role for BDNF in the survival, rather than the proliferation or differentiation, of adult-born neurons (e.g., [167]).

1.4 Molecular mechanisms of learning and memory in the olfactory system

1.4.1 Molecular mechanisms in the OB

Intrinsic memory mechanisms within the OB appear to share common pathways and adhere to similar pharmacologically-elaborated phases as have been elucidated in IA-based neural plasticity and memory studies. For example, as observed in the hippocampus after IA conditioning, PKA levels in the neonatal OB increase shortly after odor conditioning, blocking PKA activation in OB disrupts LTM but not STM, and exogenous administration of PKA into the OB ten minutes after conditioning enhances LTM persistence [71]. Odor-reward conditioning, but not pseudoconditioning, induces increased CREB activity in neonatal OB mitral cell nuclei 10 minutes after training, suggesting that CREB-related plasticity in mitral cells may be important for formation of odor LTM [135]. The MAPK/extracellular signal-related kinase (ERK) pathway is also activated by odor learning in neonates; odor stimulation induced ERK phosphorylation in selective populations of OB neurons related to the identity of the learned odor [139]. Neonatal odor learning, like hippocampal LTP, appears to rely on NMDA receptor activation and the increased expression of synaptic AMPA receptors [45, 112]; notably, the PKA-dependent phosphorylation of AMPA receptor sub-unit GluA1 rises with a similar timecourse as CREB levels in mitral cells, peaking at about ten minutes post-conditioning [45]. BDNF mRNA levels increase in the OB and piriform cortex within two hours of olfactory fear learning [89]. To the extent that a substantially common set of essential molecular mechanisms is employed, the important distinctions between one-trial learning and appeti-

tive statistical learning become within which neurons, under what conditions, to what extent, and *when* these mechanisms are invoked.

Most studies of olfactory learning and memory that have measured the form of the odor memory (typically via generalization gradients) have been performed in adult animals and at STM timescales. There is little research to date on the molecular mechanisms underlying bulbar STM, though there is a substantial literature on the effects of neuromodulators, hormones [54], and other extracellular signaling molecules. Noradrenergic effects within OB have been studied both in non-associative and associative olfactory representational learning studies (reviewed in [116]). Indeed, bulbar NE may be essential for even the simplest forms of odor learning; notably, a nonspecific infusion of NE into OB suffices to restore the non-associative learning deficits arising from depletion of cortically-projecting NE fibers [73], though bulbar NE levels induced by moderate stress also can suppress OB-dependent STM in some contexts [127]. In neonatal rats, bulbar NE is necessary for odor learning; indeed, the rapid odor learning exhibited by neonates is facilitated by the heavy release of NE into OB during learning owing to a hyperfunctional locus coeruleus [146, 176, 177]. Other classical neuromodulators, notably acetylcholine acting at muscarinic receptors within OB, also exert effects within OB circuitry on odor learning and STM maintenance [51, 52].

BDNF effects with OB are of particular interest in the first two chapters of this dissertation. BDNF is clearly implicated in LTM formation for IA learning; while it has been much less thoroughly studied in the olfactory system, BDNF transcription is activated in OB and piriform cortex after odor conditioning [89], and olfactory sensory deprivation reduces BDNF expression in the neonatal OB

[134]. BDNF and its precursor proBDNF each exert distinct physiological effects on OB neuronal excitability and plasticity [130]. BDNF heterozygous knockout mice and Val66Met point mutants exhibit reduced activity-dependent secretion of BDNF. Both mutants habituate normally to odors but exhibit greatly reduced spontaneous discrimination [13], suggesting an impairment in their ability to form specific odor representations. The clearest effects of BDNF on the OB, however, are structural in nature, substantially affecting dendritic arborization and adult neurogenesis.

1.4.2 Structural mechanisms in the OB

Long-term potentiation. Long-term potentiation has been clearly if sparsely observed in the early olfactory system, notably within piriform cortex and its ascending synapses into OB. NMDA receptor-dependent LTP has been demonstrated at afferent and associative fiber synapses within piriform cortical slices, and coactivation of the two can facilitate a form of associative LTP if local inhibition is suppressed [94, 95]. Piriform pyramidal neuron feedback projections onto OB granule cells also exhibit spike timing-dependent LTP [67], which may be a particularly powerful computational element given the importance of dynamical, timing-dependent interactions within OB circuitry. Contemporary models of OB-piriform computations have regarded these circuits as a pattern separation/completion network not unlike the dentate gyrus/CA1 circuit of hippocampus, in which piriform association fibers underlie pattern completion [12, 76] and their feedback projections onto inhibitory granule cells within OB underlie pattern separation [174], within a common recurrent circuit. This rich and structured plasticity requires further experimental and theoretical de-

velopment, but exemplifies the capacities of the olfactory system as a model for understanding complex memory systems.

Neuronal and Synaptic Morphology. Spine densities in OB and piriform cortex are affected by odor learning and by learning-associated trophic factors, notably BDNF. In piriform cortex, spine density on pyramidal neurons increased in odor-conditioned rats compared with pseudoconditioned or naïve controls, an effect potentially corresponding to increased synaptic weights in the association fiber network [101]. In the neonatal OB, dendritic branching and spine morphology is substantially regulated by BDNF signaling mediated by the TrkB receptor [78, 131]. In adults, TrkB receptor expression in the OB persists [123], and BDNF continues to regulate OB dendritogenesis, among parvalbumin-expressing neurons of the external plexiform layer [19]. More recently, [133] found that *bdnf* overexpression in the granule cells increased dendritic spine density. In combination with the integration of adult-born neurons into the OB network (discussed below), it is clear that the regulation of dendritic connectivity among OB neurons is a significant determinant of OB functional plasticity, and that BDNF is a crucial regulator of the underlying mechanisms.

Adult Neurogenesis. Adult neurogenesis within in the OB has been studied extensively with regard to its effects on, and mediation of, odor learning and memory. The differentiation of adult-born neurons within OB and its relevance for olfactory perception and odor learning have been extensively studied and reviewed elsewhere [111, 109, 69]. Of particular interest for present purposes, though, is the regulation of these neuronal differentiation processes by signaling molecules and other established mediators of olfactory learning, as well as

timing and task dependencies that may suggest points of particular mechanistic importance.

The incorporation of new neurons is most widely associated with olfactory LTM; as described above, the selective ablation of newly differentiated OB neurons specifically disrupted a long-term odor memory [4]. However, there also are indications that adult-born neurons may participate in STM processes. Infusions of the antimitotic drug AraC into the lateral ventricle of rats abolished the arrival of new neurons into the OB, while largely sparing hippocampal neurogenesis, and impaired short-term nonassociative memory for odors learned 20-28 days thereafter [30]. Specifically, the absence of new neurons had no significant effect on a 30-minute memory for a habituated odor, but 60-, 90-, and 120-minute odor memories were disrupted compared with control animals. Critically, and in sharp contrast, AraC treatment did not have any effect on olfactory *associative* memory, when a conditioned odorant was paired with reward over four days, and odor memory was tested 24 hours and 1 week after the end of training.

Interestingly, it has been proposed that non-associative and associative odor learning preferentially activate neurons of different ages within OB [16]. Specifically, non-associative perceptual learning preferentially activated newly-arrived neurons (~2 weeks old), as measured by c-fos immunoreactivity, whereas water-rewarded odor discrimination training in a go/no-go task preferentially activated more mature, though still recently generated, interneurons (5-9 weeks of age). This result is entirely consistent with the results described above, in that the OBs of AraC-infused mice in that study were devoid of neurons younger than 3-4 weeks, as required for non-associative odor learning, but possessed a full complement of neurons in the 5-9 week age range, as were most heavily

utilized in the rewarded task. (Also of potential interest is that activation does not necessarily correspond to increased survival; olfactory go/no-go training has been associated with enhancing the survival of 2-4 week old neurons in OB, while increasing apoptosis in 5-week old neurons, and not affecting fully mature interneurons 9 weeks of age or older; [147]). These results still beg the question, of course, of what factors in these different training paradigms underlie the selective recruitment of different cohorts of new neurons. These results illustrate another advantage of the olfactory system for studies of complex and naturalistic learning, in which task parameters may determine the differential utilization of OB (and non-OB [121]) circuit elements for odor-dependent learning.

BDNF signaling is a significant contributor to the survival of new neurons in the OB. BDNF levels are similar in both the site of neurogenesis in the SVZ and in the OB, the target of migration, and regulate both neuronal migration and differentiation (the latter via the MAPK pathway) [154]. Infusions of BDNF into the lateral ventricle of adult rats significantly increased the generation and/or survival of adult-born neurons in the OB [17, 198]; in an analogous *in vitro* study, BDNF administered to SVZ-derived neuroblasts from rat promoted their survival [100]. Mice heterozygous for either the BDNF gene or its TrkB receptor exhibit reduced neuron survival in the OB, as do mice with the Val66Met point mutation in the BDNF gene, which impairs activity-dependent BDNF secretion [13]; these mutants also exhibited impaired nonassociative odor learning as described above. Neuronal proliferation was not affected by these mutations, suggesting that the effects of BDNF primarily relate to survival and differentiation. The powerful effects of this neurotrophin on olfactory learning and neuronal differentiation, and its association with established learning-associated molecular cascades, render it a strong candidate for study in order to elucidate

the complex relationships underlying these representational, statistical learning processes in naturalistic contexts.

1.5 In summary

Understanding the neurophysiological basis of natural learning and memory is one of the great challenges of neuroscience. Much of what is known about the molecular mechanisms underlying learning derives from one-trial learning paradigms of inhibitory avoidance (fear conditioning), though research in other plastic neural systems has indicated that they share many, though not all, of the same underlying molecular and structural mechanisms of plasticity. One-trial odor learning studies, which induce plasticity in olfactory bulb, suggest that these cortical circuits also rely on these common mechanisms for plasticity – although bulbar memory also depends on adult neurogenesis, a structural mechanism which it shares only with the hippocampus.

Most natural learning, however, is less categorical than these one-trial paradigms, requiring multiple encounters in order to elucidate relevant stimuli and learn appropriate associations. Appetitive learning in adults, for example, tends to be gradual, conditional, and statistical in nature. This raises new mechanistic questions: how does learning accumulate over multiple trials? How do STM and LTM mechanisms interact over the extended timescales of natural experience? How does learning change the form, or quality, of a sensory representation in response to accumulating information? What is the role of temporal specificity of these mechanisms in memory for incrementally acquired information? In the remaining chapters, I describe three studies that aim

to answer these questions in greater depth.

CHAPTER 2

**NEUROTROPHIN RECEPTOR ACTIVITY IN THE OLFACTORY BULB IS
NEEDED FOR LONG-TERM, BUT NOT SHORT-TERM, OLFACTORY
MEMORY**

2.1 Abstract

It is well established that, following one-trial learning, long-term memory (LTM) consolidation requires protein synthesis whereas short-term memory (STM) does not. A great deal of work has examined the role of specific protein products in the consolidation of one-trial learning. What remains unclear is the differential involvement of plasticity-related mechanisms, including neurotrophins like brain-derived neurotrophic factor (BDNF), in STM and LTM for multi-trial learning. In this study, mice were trained over several trials to learn an odor-reward association and memory for the association was tested 2 or 48 hours later. Mice were given OB-specific infusions of tyrosine kinase receptor antagonist, K252a, immediately prior to training. We found that mice given K252a did not differ from controls in learning rate, but showed impaired memory when tested 48 hours, but not 2 hours, after training. This LTM deficit in the drug group was associated with lower selectivity for digging in the rewarded odour. The study shows that neurotrophin receptor activity in the OB is needed for LTM of incrementally-acquired information, but not STM.

2.2 Introduction

Definitions of short-term memory (STM) and long-term memory (LTM) have varied among fields of research. Studies examining molecular mechanisms have shown that memories lasting longer than 24 hours rely on protein synthesis, whereas those lasting up to 6 hours do not [28, 48, 53, 82]. These two durations, called LTM and STM, respectively, are defined by their different dependencies on protein synthesis. Importantly, findings from these studies have shown that STM and LTM rely on distinct intracellular pathways from the outset.

Most of this foundational work, including attempts to elucidate the role of specific plasticity-related proteins, has been done using one-trial learning paradigms. Multiple studies [29, 74, 84] have shown clear dissociations between the various mechanisms involved in STM and LTM for these paradigms. Relevant to the present experiment, blockade of the neurotrophin, brain-derived neurotrophic factor (BDNF) signaling through its receptor, TrkB, or through function-blocking anti-BDNF, has been shown to disrupt LTM but not STM for a one-trial IA event [35]. BDNF has a demonstrated role in many forms of long-term structural plasticity (LTP, adult neurogenesis, dendritic arborization, [19, 78, 103, 131, 167, 169]) and, thus, its activity is likely necessary for LTM consolidation. In fact, infusions of recombinant BDNF into the HPC rescued IA memory from amnesia induced by glucocorticoid receptor blockade [35].

However, generalization of the findings to incremental, or multi-trial, learning can be problematic. In one-trial paradigms, the learning event is somewhat discrete in time. Thus, temporal patterns of molecular activity following the learning event can be ascertained with reasonable specificity. One obvious issue

is that incremental learning is, by definition, temporally distributed. As such, what constitutes the “post-learning” period is ambiguous and difficult to ascertain experimentally, and causes problems for the study of temporal patterns of molecular activity in memory. While this is can be seen as a major limitation of multi-trial paradigms, we suggest that it instead constitutes an important research question that warrants investigation.

The limited research looking at the timecourse for plasticity-related activity after multi-trial learning suggests that these mechanisms do differ meaningfully from one-trial learning. Mizuno and colleagues [143, 142] trained rats for multiple days on a radial-arm maze task. The researchers measured protein levels in the HPC for active CREB and PKA. In contrast to a one-trial, IA task where CREB/PKA activity in the HPC peaked immediately after the initial exposure [22], Mizuno and colleagues [142] found detectable levels of CREB and PKA 15 minutes after training but only after eight days of training. Importantly, this finding cannot be simply explained by an accumulation of learning, since performance increases across days, suggesting memory retention between days occurs with no corresponding changes in CREB or PKA activity. More specific to BDNF, Mizuno and colleagues [143] found that BDNF mRNA increased 15 minutes after multi-trial, radial-arm maze training only after 28 days. On the 28th day of training, researchers began giving continuous infusions of anti-sense BDNF oligonucleotides (ASO). After 4 days of the infusions, animal performance on the task returned to initial levels. The results demonstrate that BDNF activity after 28 days of training is needed for memory persistence.

Together, these findings strongly show that some characteristics of the molecular mechanisms involved in memory for multi-trial learning differ from

one-trial learning. However, these differences are under-explored. As a first step, we aim to determine a mechanistic dissociation for BDNF receptor activity between STM and LTM pathways in incremental learning. We do this using an olfactory associative discrimination task. Mice received OB-specific infusions of a BDNF receptor blocker prior to training of an odor-reward association over 20 trials. Animals were then tested 2 hours (STM) or 48 hours (LTM) after training. We found that animals infused with the receptor blocker showed lower LTM compared to controls, but normal STM. We also found that LTM deficits were associated with lower certainty of the odor-reward association in the drug-infused group.

2.3 Materials and Methods

2.3.1 Animals

A total of 27 adult male CD-1 mice (Charles River) were used in this experiment. The mice were 8 weeks old at the beginning of the experiment. All procedures were performed under the auspices of a protocol approved by the Cornell University Institutional Animal Care and Use Committee (IACUC). Cornell University is accredited by The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).

The mice were kept on a 12:12 hour reverse light/dark cycle and allowed free access to water at all times. They were kept on a food-restriction schedule designed to keep them around 90% of their free feeding weight for the duration of the behavioral experiments. This food restriction schedule began 3 days

before the beginning of behavioral tasks.

2.3.2 Olfactory bulb cannulations

Mice were anesthetized with gaseous 4% isoflurane (Henry Schein, Dublin, OH, USA) in pure oxygen and secured into a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). For the duration of the surgery, mice were kept under 1.5-2% isoflurane anesthesia through a nose cone. Guide cannulae (26-gauge; PlasticsOne, Roanoke, VA, USA) were inserted into both OB using the following coordinates with respect to bregma: AP +5.0mm, ML +/-0.75mm, DV 1.0mm. Two screws were drilled into the skull over the cerebellar formation to provide an anchor for the dental cement cap. Dental cement was used to secure the guide cannulae to these screws and to cover the incision area. Dummy plugs were placed into the guide cannulae to prevent blockage and infection. For two days after the surgeries, mice were fed softened food and given injections of an analgesia, ketoprofen (0.2mg/kg mouse), and saline. Animals recovered for at least 7 days before beginning behavioral training.

2.3.3 Associative discrimination task

Apparatus.

Animals were tested in a clear Plexiglass cage (28 cm long x 17 cm wide x 12 cm high) with a removable black opaque center divider. Animals were placed into the resting chamber, at the beginning of each session. One trial included

the lifting of the divider, animals entering the test chamber, and returning to the resting chamber.

Infusions.

Animals received OB-specific infusions of tyrosine kinase receptor inhibitor, K252a (Sigma-Aldrich K2015; 5% DMSO in saline) or a vehicle (saline with 5% DMSO). Drug or vehicle was delivered bilaterally into the OB at a volume of 2.0 uL and 0.2 uL/min (so the total time of the infusion was 10 minutes). Mice were not anesthetized during the infusions. Injectors were left inside cannulae for an additional 5 minutes to allow diffusion of the remaining liquid. Mice received infusions immediately before training only. In a control experiment, mice were given infusions only prior to testing.

Odor sets.

For shaping, we used (+/-)limonene (Sigma-Aldrich, St. Louis, MO, USA) as the rewarded odor and mineral oil as the unrewarded odor. For the training and testing phases, four sets of odor pairs were used: butanoic acid, pentanoic acid; butyl butyrate, propyl butyrate; 2-octanone, 2-heptanone; and pentanol, hexanol. All odorants were diluted in mineral oil so as to emit a theoretical steady-state vapor phase partial pressure of 1.0 Pa [41]. Each odor set consisted of two odors with the same functional group that differed from each other by one carbon-chain length. These pairs are considered perceptually similar [41, 42]. For training and testing, one odor of each set was chosen as the rewarded odor and the other was unrewarded. This assignment was counterbalanced to

avoid odor preference biases.

Shaping.

Animals underwent a ten-day behavioral shaping period prior to the start of the experiment. Animals were brought to the experiment room and handled for 10 minutes per day for the first two days after recovery from surgeries (Day 1 and 2). On Day 3, a petri dish (Pyrex, 60 mm diameter, 15 mm height) of play sand (Quikrete; Atlanta, GA) scented with (+/-)-limonene was placed into the home cages of the animals. These dishes were filled with 10-15 5-mg sucrose pellet reward (PJ Noyes Precision Pellets; TestDiet, Richmond, IN). The sand and sucrose were replenished on Day 4. On Days 5-6, animals were acclimated to the behavioral apparatus. Two dishes of scented sand, limonene and mineral oil, were placed into the Plexiglass chamber without the center divider. Ten sugar pellets were mixed into the limonene-scented dish at various depths. Animals were placed into the test chamber for 10 minutes. They were allowed to freely explore the chamber and consume the sugar pellets.

On Day 7, animals were introduced to a shortened version of the final testing procedure. Again, two dishes were placed into the behavioral apparatus, including the center divider. A single sugar pellet was placed on top of the limonene-scented sand. The animals were placed into the resting chamber. The center divider was lifted and animals were allowed to enter the test chamber and retrieve the sugar pellet. Animals were either ushered back into the resting chamber when they retrieved the sugar pellet or after 5 minutes elapsed. This was repeated for 10 trials. On a given trial, the dishes were randomly placed on the left or right using a random number generator. The whole procedure was

repeated on Day 8. Crucially, on Day 8, the sugar pellet was buried progressively deeper with each trial. Animals were digging for an unseen sugar pellet by Trial 10 on Day 8.

On Day 9, animals were presented with the full 20-trial version of the task. On this day, from Trial 1, sugar pellets were fully buried under the sand in the dish. A trial lasted only 1 minute. On this day, animals were allowed to dig freely in both dishes for the reward. On Day 10, the animals underwent the same 20 trials, but they were not allowed to self-correct. That is, if the animals dug in the mineral oil first, they were ushered back into the resting chamber and the next trial began. If animals dug in the limonene first, they were allowed to retrieve the sugar reward before being ushered back into the resting chamber.

Training and Testing.

Once animals were shaped and able to dig for an unseen sugar reward, we began the experiment formally. The training phase began two days after shaping was completed. To test the main hypothesis, infusions were only given prior to training. For the retrieval control, infusions were given immediately before testing, not training. Training began immediately following infusions. The animals were placed into the resting chamber. Two dishes of sand scented with a novel odor pair (Table 1) were placed in the test chamber. The sugar pellet was buried fully in the rewarded odor dish. Training included 20 trials, one minute per trial. If the animal dug in the unrewarded odor first, they were ushered back into the resting chamber and the next trial began immediately. If animals dug in the rewarded odor first, they were allowed to retrieve the sugar reward, ushered back into the resting chamber and the next trial began immediately.

Animals were tested either 2 hours (STM) or 48 hours (LTM) after training. The procedure and odors were the same as in training.

For both training and testing, sugar was omitted from the dishes on Trials 1, 5, 10, 15, and 20. Digging time in each of dishes was recorded over the course of 1 minute. On these trials, animals were allowed to dig freely in either dish, including self-correction. At the end of the minute, if the animal had dug in the rewarded odor first, a sugar pellet was surreptitiously dropped into that dish. If the animal dug in the unrewarded odor first, it was ushered back into the resting chamber without reward.

Data analysis.

Analyses were performed on two dependent measures: average proportion correct and Selectivity Index (SI). On a given trial during training and testing, 1 was assigned to trials in which the mouse dug in the rewarded odor first. If the animal dug in the unrewarded odor first, the trial was given a 0. We averaged every five trials to create a proportion correct measure across four trial blocks (e.g. Trial Block 1 is the average of Trials 1-5, Trial Block 2 is the average of Trials 6-10, and so on). The SI was computed by dividing the difference between digging times in the rewarded odor and the unrewarded by the sum of those times. Thus, a SI close to 0 means the animals did not dig preferentially in one odor and a SI close to 1 represents high preference for digging in the rewarded odor. SIs were computed only for Trials 1, 5, 10, 15, 20 of training and testing.

We performed linear mixed effects analyses on these transformed measures using IBM SPSS 22.0 for all analyses. The dependent measures are not contin-

uous and unbound variables and violate two assumptions for linear models. Thus, we performed a logit transformation prior to statistical analysis. We first replaced all values of 0 with 0.01 and values of 1 with 0.99. The final transformed variable, Y, is computed using Eq. (2.1).

$$Y = \ln\left(\frac{X}{1 - X}\right) \quad (2.1)$$

where X is value-replaced dependent variable.

Mixed effects models are similar to the well-known repeated measures analysis of variance (ANOVA) tests, however they are better suited for data sets with a hierarchical structure (e.g. differences in observations across time between multiple groups). The fixed effects differed for each analysis, the specific effects are described along with the Results below. Crucially, in all the analyses, we included random effects for individual mouse and odor sets nested within mouse. The random effects account for variance introduced by non-experimental factors, such as baseline odor preferences by individual mice. We used estimated marginal means to perform post-hocs on significant interactions from the full model. A Bonferroni correction for multiple comparisons was used for post-hoc pairwise comparisons.

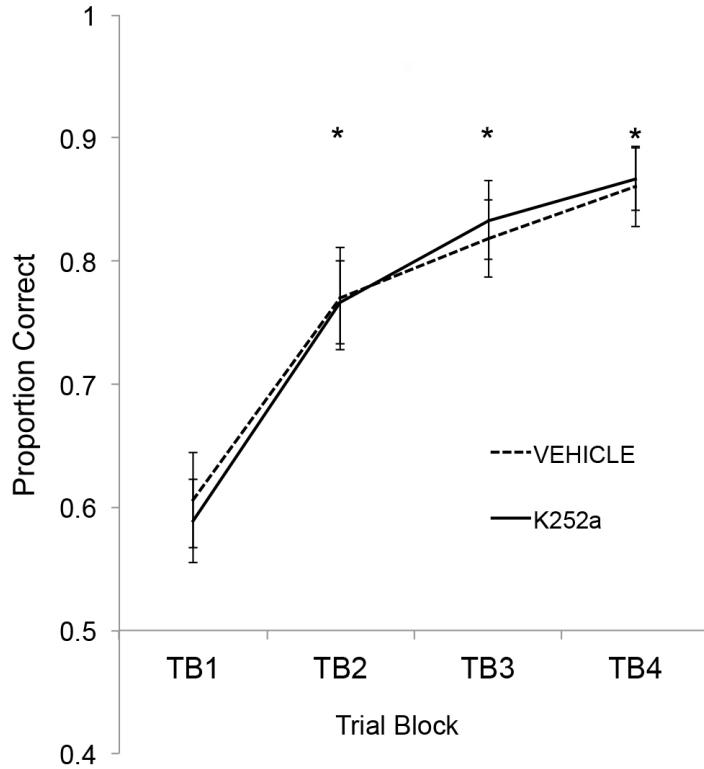


Figure 2.1: K252a infusion did not affect learning rate. The figure shows the increase in proportion correct across initial Training for both drug and vehicle groups. TB2, TB3, and TB4 were significantly higher than TB1 for both K252a and Vehicle groups ($p < .001$ for all comparisons, except between TB1 and TB2 for K252a where $p = .001$)

2.4 Results

2.4.1 Vehicle and K252a-infused mice show no differences in rate of acquisition

First, we analyzed the learning curves for the training phase to assess the effect of the infusions on the rate of acquisition. We ran a linear mixed model with two

fixed effects, infusion and trial block (TB). Mouse and odor set nested within mouse were random effects in our model. We observed a significant main effect of TB ($F(3, 183.692) = 43.735, p <.001$) and no significant interaction of infusion and TB ($F(3, 183.692) = .111, p = .954$), showing that K252a and vehicle groups did not differ in learning rate.

Post-hoc tests, using the Bonferroni adjustment for multiple comparisons, confirmed that drug and vehicle groups did not differ on any of the trial blocks ($p >.05$ for all comparisons). In addition, TB2, TB3, and TB4 were significantly higher than TB1 ($p <.001$ for all comparisons, except between TB1 and TB2 for K252a where $p = .001$) for both K252a and vehicle groups (Figure 2.1) showing that all animals successfully learned the association. Importantly, the results show that K252a infusions did not affect the rate of acquisition of the odor-reward association.

2.4.2 Mice infused with K252a showed deficits in long-term, but not short-term, memory

In these analyses, we addressed our central question of the differential reliance on BDNF receptor activity between STM and LTM pathways. We ran a linear mixed model with three fixed effects: training/testing, STM/LTM, infusion group. Again, mouse and odor set nested within mouse were random effects. Our linear mixed model analysis showed a significant 3-way interaction between training/testing, infusion, and STM/LTM ($F(1,60.916) = 5.025, p = .029$). No other main effects or interactions were observed.

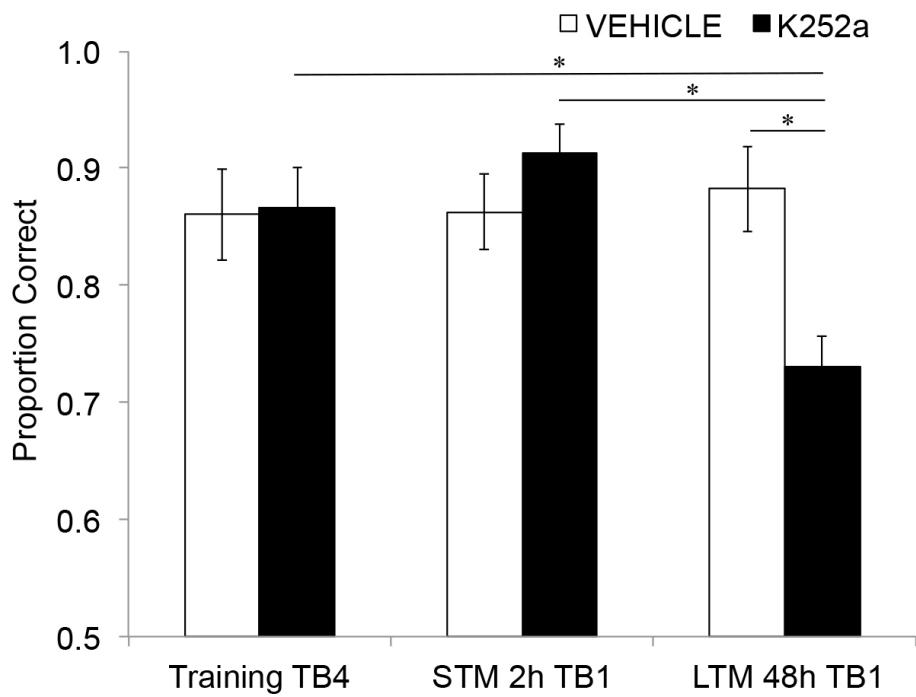


Figure 2.2: LTM deficits found in K252a-infused group alone. The first Trial Block (TB1) of 48-hour testing was significantly lower than TB4 of training ($p = .003$) for the drug group but not vehicle group ($p = .839$). For the drug group, TB1 was significantly lower for LTM than STM ($p = .027$) and significantly lower than the vehicle group at LTM ($p = .009$).

Because we are interested in differences in retention between training and testing periods, we compared the TB4 of training with the TB1 of testing. Post-hoc pairwise comparisons with the Bonferroni adjustment showed that TB1 for the drug group was significantly lower during LTM than for TB4 of training ($p = .003$), but there was no significant difference between TB4 of training and TB1 of LTM for the vehicle group ($p = .839$). This shows that while the vehicle group retained LTM from the training phase, the drug group did not. Furthermore, there were no differences in proportion correct between TB1 of STM and TB1 of LTM for the vehicle group ($p = .317$). For the drug group, however, TB1 was

significantly lower for LTM than STM ($p = .027$). Additionally, TB1 for the drug group was significantly lower than the vehicle group at LTM ($p = .009$), but not at STM ($p = .381$). All together, the results strongly demonstrate that the presence of K252a in the OB around the time of learning has an amnesic effect on LTM, but not STM (Figure 2.2).

2.4.3 K252a-infused animals show lower selectivity for rewarded odor during LTM testing

Whereas the proportion correct measure (Figure 2.2) allowed us to assess the accuracy of the decision to dig in the scented dishes, the Selectivity Index (SI), which is based on digging time measurements, in part allowed us to access the certainty with which those digging decisions were made. We computed a SI for trials 1, 5, 10, 15, and 20 (called probe trial below) of training, STM, and LTM. The linear mixed model analysis yielded significant main effects of training/testing ($F(1, 500.794) = 75.213, p <.001$) and trial period ($F(4, 466.497) = 42.601, p <.001$), showing that the mean SI differed between testing and training phases, and that the SI across all groups differed between T1-T20. We also observed a significant 4-way interaction between the four fixed effects: training/testing, STM/LTM, infusion and probe trial ($F(4, 466.864) = 3.036, p = .017$), and no significant 3-way interactions ($p >.05$ for all). There was a significant 2-way interaction between training/testing phase and probe trial ($F(4, 466.975) = 16.908, p <.001$) indicating that the SI across the probe trials (T1, T5, T10, T15, and T20) differed between the training and testing phases. There were no other significant main effects or interactions.

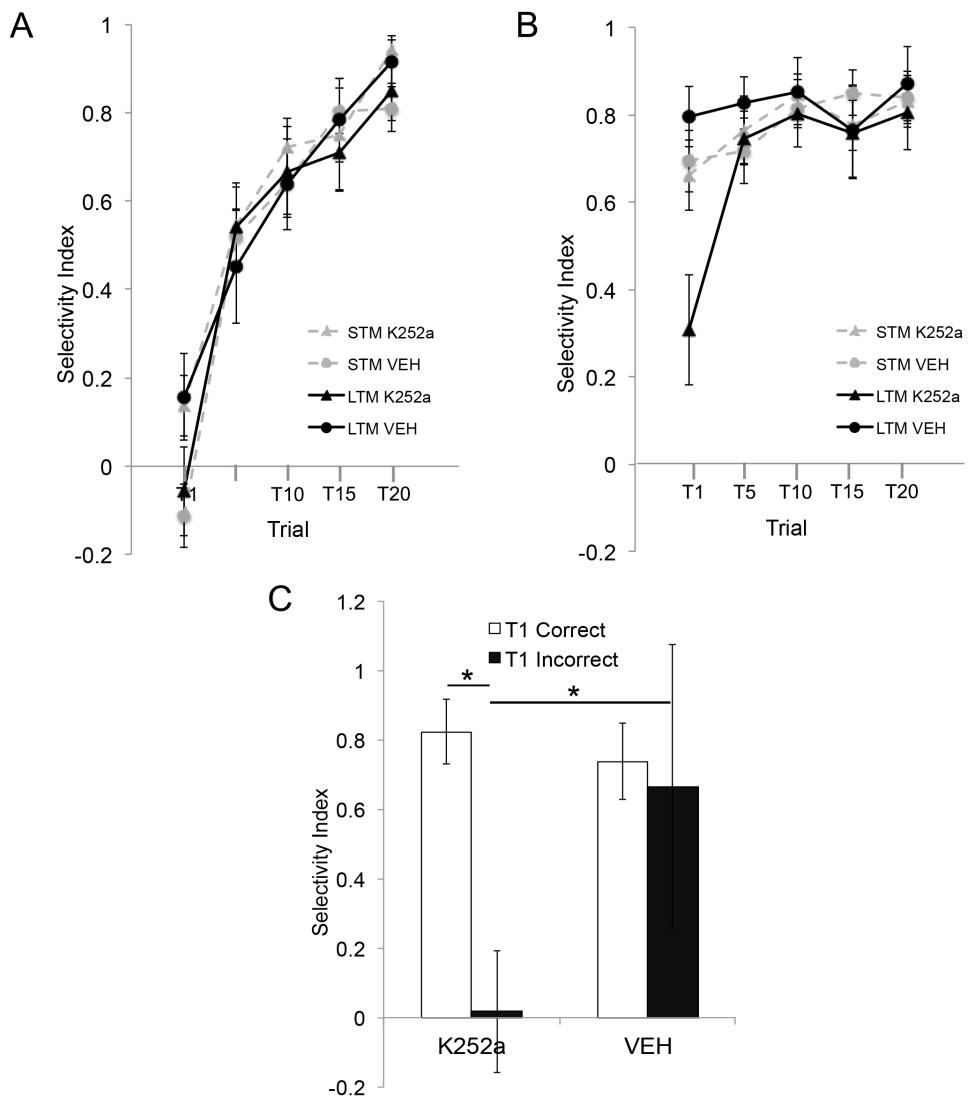


Figure 2.3: **(A)** During initial Training, all groups increased in selectivity for rewarded odor across the training trials. **(B)** During STM (2 hours after Training) testing, both K252a and Vehicle groups retained selectivity for rewarded odor from the very first trial and this persisted. During LTM (48 hours after Training) testing, the K252a group trended toward lower selectivity for the rewarded odor ($p = .064$). **(C)** On Trial 1 of LTM testing, K252a-infused animals had a significantly lower SI than the vehicle group when the trial was incorrect ($p < .001$), but not when it was correct ($p = .663$). Furthermore, the drug group had a significantly lower SI for incorrect T1 than correct T1 ($p = .001$).

Post-hoc tests using the Bonferroni adjustment (Figure 2.3B) showed that, during LTM testing, the SI on T20 for the drug group was significantly higher than T1 ($p = .010$) suggesting that learning occurred during testing whereas it did not for the vehicle group ($p = 1.000$). It was surprising then that key post-hoc comparisons showed that during LTM testing, the difference between the SI on T1 for the drug group and the vehicle group was marginally significant ($p = .064$). In this analysis, however, the SI is the average of SIs from both correct (the animal dug in the rewarded odor first) and incorrect (those where mice dug in the incorrect odor first) trials. Differences between groups could be masked through this averaging if the drug effect was a function of correct decisions. To explore this further, we divided the probe trials into correct and incorrect trials and then compared the SIs between the drug and vehicle group for STM and LTM.

We performed a linear mixed model with 4 fixed effects: infusion, STM/LTM, correct/incorrect, and probe trial (T1, T5, T10, T15, and T20). Mouse and odor set nested within mouse were random effects. We found a main effect of correct/incorrect ($F(1, 259.580) = 4.704, p = .031$) suggesting that on average the SIs for correct and incorrect trials differed. We found a significant 3-way interaction of infusion, STM/LTM, and correct/incorrect ($F(1, 225.120) = 6.422, p = .012$); significant 2-way interaction of infusion and correct/incorrect ($F(1, 249.682) = 4.969, p = .027$), as well as, a 2-way interaction of infusion and STM/LTM ($F(1, 178.020) = 4.147, p = .043$).

Pairwise comparisons using the Bonferroni correction showed that, during STM testing, SIs were not different between correct and incorrect trials for T1, T5, T10, T15, or T20 ($p > .05$ for all). This was true for both drug and vehicle

groups. For LTM testing, where the proportion correct data (Figure 2.2) showed poor memory in the drug group, we found that K252a-infused animals had a significantly lower SI than the vehicle group when T1 was incorrect ($p < .001$), but not when T1 was correct ($p = .663$). Furthermore, the drug group had a significantly lower SI for incorrect T1 than correct T1 ($p = .001$). Figure 2.3C shows the comparisons for T1 during LTM.

In summary, we suggested that the marginal significance for the difference between SI on T1 during LTM test (Figure 2.3B) was perhaps due to an averaging of correct and incorrect trials which masked the difference. Indeed, when we compared the selectivity index as a function of correct and incorrect digging decisions, we found that during T1 of LTM testing, the K252a-infused group showed no digging preference on trials where it dug in the unrewarded odour first whereas the vehicle group dug with high selectivity for the rewarded odour regardless of the correctness of their decision. In a separate analysis, we compared the total digging times across the drug and vehicle groups during the first trial of LTM testing and found that the digging times did not differ between the drug and vehicle groups on this T1 of LTM ($p = .094$) suggesting that animals did not differ in their motivations to dig. The results suggest that animals given K252a had a lower LTM performance due to more uncertainty of the rewarded odour as measured by digging selectivity. Taken together, the findings suggest that K252a infusions disrupted OB-specific, neurotrophin pathways that underlie memory mechanisms for distinguishing between the rewarded and unrewarded odors.

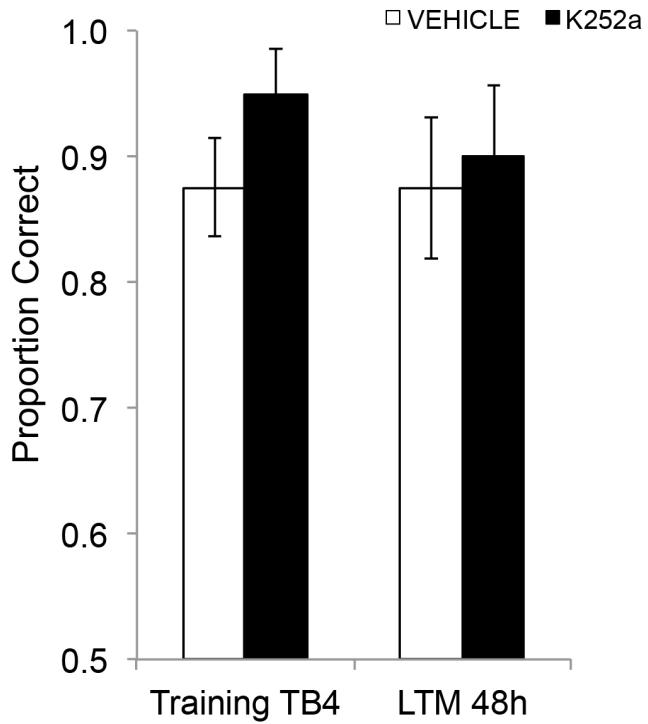


Figure 2.4: When infusions were given prior to 48-hour LTM testing, instead of prior to Training, no memory differences were observed between the groups ($p > .05$ for all main effects and interactions).

2.4.4 K252a does not affect memory retrieval

Finally, in order to make sure that the LTM deficits in the drug group were not due to later effects of the infusions on memory retrieval, we ran a separate control where animals received infusions of vehicle or K252a prior to LTM testing (Figure 2.4). No infusions were given prior to training. We ran a linear mixed model with infusion and training/testing as fixed effects. Again, mouse and odor set nested within mouse were random effects.

As with the previous proportion correct data (Figure 2.2), our analysis com-

pared TB4 of training with TB1 of testing. We found no significant main effects of training/testing ($F(1,14) = .055, p = .817$) or infusion ($F(1, 14) = 1.361, p = .263$) and no significant interaction between training/testing and infusion ($F(1,14) = .592, p = .454$). This analysis, in conjunction with our main results, suggests that the observed LTM deficits did not result from disruptions to memory retrieval.

2.5 Chapter 2 Discussion

The goal of this study was to demonstrate a mechanistic dissociation between STM and LTM pathways for multi-trial learning. Indeed, we found that animals infused with a tyrosine kinase receptor antagonist in the OB prior to training showed memory deficits during a 48h, but not 2h, memory test. Drug infusions prior to training did not affect learning rate and infusions prior to testing did not affect retrieval suggesting that the amnesiac effect is specific to mechanisms active around the time of conditioning. Finally, our data suggest that the observed LTM deficit in K252a-infused animals was associated with lower certainty of the odor-reward association corresponding with a low selectivity for the rewarded odor over the unrewarded. The results show that LTM for incrementally-acquired information relies on neurotrophin receptor activity, whereas STM does not. In addition, the present study represents a contrast to previous experiments which examined these mechanisms in cortical or subcortical regions, like the HPC or amygdala. The fact that we observe a STM/LTM dissociation for BDNF-TrkB pathway in the OB, a primary sensory region, implies that the dissociation is a characteristic of the nervous system at the synaptic level, rather than the product of higher-order, multi-region processing.

A limitation of the study, however, is that we were unable to ascertain which of neurotrophin/receptor pathways - including Nerve Growth Factor (NGF)/TrkA, BDNF/TrkB, and Neurotrophin-3 (NT-3)/TrkC are involved in the observed LTM effect. Based on behavioral, anatomical, and mechanistic evidence from other research, we suggest that the observed effects in this study are largely mediated by the BDNF-TrkB pathway.

Behaviorally, a study using K252a infusions into the amygdala found amnesiac effects that were specific to the TrkB receptor [160]. More importantly, a study in chicks gave intracerebral infusions of BDNF, NGF, or NT-3 ASO prior to one-trial avoidance training [88]. Researchers found that although ASO administration for each neutrophin reduced mRNA levels for each neurotrophin respectively, only chicks receiving the BDNF ASO showed a LTM deficit at 24 hours with no corresponding 1-hour STM deficit. The study strongly demonstrates that BDNF, and not NGF or NT-3, shows a STM/LTM dissociation.

Anatomically, TrkA expression is high in the olfactory epithelium (OE) and horizontal basal cells [165] and TrkC is found primarily in the olfactory sensory neurons ([165]) whereas TrkB reactivity is found in every layer of the OB [18]. Although neurons in the OE are essential for various olfactory behaviors, like odor detection [97], their role has not been shown to be essential for long-term olfactory memory. In fact, memory deficits at the observed timescales are likely due to disruptions long-lasting forms of structural plasticity, like the survival of adult-born neurons [6, 98, 164].

In a recent study [10], animals were trained over 4 days to dig for reward inside a scented dished. On the fifth day, the animals were injected with diphtheria toxin (DT) to selectively ablate the adult-born neurons that were present in

the OB at the time of learning. Animals who received the DT showed poor LTM for the rewarded odor. The authors suggest that ablation of granules cells (GCs) are responsible for this effect and indeed showed fewer adult-born granule cells in animals that underwent DT-treatment. Activity-dependent survival of adult-born neurons has been well-established to be primarily in the periglomerular cells and granule cells, where, importantly, TrkB and not the other neurotrophin receptors are expressed. Thus, mechanistically, the BDNF-TrkB pathway seems responsible for LTM in our task.

In the OB, adult-born neurons migrate down the rostral migratory stream from the subventricular zone (SVZ). A wealth of research shows the clear role of the BDNF-TrkB pathway in adult neurogenesis [13, 18, 167, 169]. However, to our knowledge, not many studies have looked at the role of the other neurotrophin pathways in OB-specific, adult neurogenesis. Fiore and colleagues [61] have shown that NGF administration can increase SVZ neuron proliferation, but this appears to co-occur with increased BDNF expression [182]. Importantly, while NGF application *in vitro* has been shown to increase the survival of SVZ-derived neurons, it appears to do this through its interaction with p75NTR and not TrkA [68]. Thus, our observed LTM deficits are likely not due to TrkA blockade or the NFG-pathway. Receptor p75NTR is not affected by K252a infusions, so if NGF activity through this receptor was involved in LTM in the present experiment, K252a infusions would not have led to observed deficits. For NT-3, the limited studies on its effects on the OB shows that NT-3 or TrkC lacking mice have no changes in the organization or size of the OB [148]. More specifically, NT-3 appears to play a role in enhancing the survival of SVZ-derived oligodendroglia, and not neurons [91].

It is alternatively possible that the observed LTM deficit is due to blockade of long-term plasticity (LTP), another canonical structural mechanism of long-term memory, in the OB. Studies have shown that both BDNF and NT-3 can potentiate excitatory synaptic transmission in the HPC [92] and inhibit GABAergic transmission [99]. Indeed, mRNA levels for both BDNF and NT-3 are upregulated following LTP-inducing high frequency stimulation in HPC slices [151]. Thus, the prevention of LTP in the OB through the TrkC/NT-3 pathway could explain our results. However, LTP has not been shown at synapses where TrkC is expressed [67, 149]. Additionally, in all cases described above, BDNF also induced similar effects making it difficult to ascertain the unique contributions of the two neurotrophins.

Thus, to the best of our knowledge, given findings from existing behavioral, anatomical, and molecular studies, we strongly suggest that the LTM deficits observed in the present study are mediated by disruptions to the BDNF-TrkB pathway in the OB, probably through the inhibition of memory-specific survival of adult-born neurons. While the dominant idea is that LTM relies on the survival of adult-born GCs [10, 69, 98, 109, 111], we acknowledge here that recent findings from Bergami and colleagues [18] suggest that TrkB-mediated signaling is primarily responsible for the survival of adult-born periglomerular cells (PGCs), not GCs. The authors show, using conditional ablation of full-length TrkB receptors specifically in adult-born neurons and long-term genetic fate mapping, that TrkB deletion decreased the survival of dopaminergic neurons, leading to a reduction in the overall number of adult-born PGCs, but not of adult-born GCs. It appears then that the involvement of the BDNF-TrkB pathway specifically in adult-born neuron survival and LTM requires further investigation. Future studies combining behavioral paradigms and cellular-level analysis are needed

to understand the unique contributions of PGCs and GCs survival to long-term olfactory memory.

Another possible explanation for the LTM deficits in our study is that we are disrupting every form of structural plasticity. Recent work by McDole and colleagues [133] shows that, *in vivo*, over-expression of BDNF, specific to the OB, led to increased GC spine density in adult mice. Closer analysis of the morphology showed that BDNF over-expressed mice had more mature spines on their apical dendrites in the distal and proximal apical, where GCs typically receive excitatory inputs. These inputs, especially from centrifugal fibers from primary olfactory cortex, have been shown to play a role in the selective apoptosis of GCs, at least in the postprandial period [102]. Thus, it's entirely possible that the action of the BDNF/TrkB pathway in the OB underlies the coordinated action of multiple forms of structural plasticity, including LTP, the survival of adult-born neurons, and dendritic growth, that together gave rise to observable LTM behaviors. Future studies that explicitly study more than one form of structural plasticity following BDNF activity manipulation, both *in vitro* and *in vivo*, are needed to truly understand the role of this pathway in the coordination of structural mechanisms of LTM.

In summary, the present study showed that LTM, but not STM, for multi-trial learning putatively relies on activity of the BDNF-TrkB pathway in the OB. This finding opens the door to important research that contributes to our fundamental understanding of memory consolidation for all types of events, not just brief, highly surprising ones. In contrast to findings from one-trial learning paradigms, incremental learning paradigms, like the olfactory discrimination task, allow us to examine the molecular mechanisms involved in the consolida-

tion of temporally distributed learning events. In addition, multi-trial learning paradigms will allow for more direct studies of the shape of memory representations and the role of molecular mechanisms in memory specificity.

CHAPTER 3

**THE ROLE OF THE BDNF-TRKB PATHWAY IN ODOUR
REPRESENTATION SPECIFICITY**

3.1 Abstract

In this chapter, we outline a set of experiments to test that memory deficits resulting from TrkB receptor blockade are primarily due to a failure to consolidate high-specificity representations. We discuss the implications of this work for our understanding of the role of the BDNF-TrkB pathway in memory consolidation, as well as, for the need to began assessing the molecular mechanisms of representational learning.

3.2 Chapter 3 Introduction

When we consider olfactory memory at the representational level [128], the differences we observe in memory performance can be said to arise from odor representations [38] that differ in strength, or in specificity, or both. For example, animals can show poor memory performance as a result of a representation with decayed strength that maintains its specificity (a human equivalent may be: “I sort of remember it being grapefruit juice and not orange juice”), or as a result of disruptions to the specificity of the memory representation that do not influence the strength of the memory (or e.g., “I am absolutely certain that it was some kind of citrus, but I don’t know which”). Being able to ascertain which of these scenarios underlie the memory deficits that we observe using traditional meth-

ods can provide much-needed insight into the role of plasticity-related mechanisms in modifying memory representations (That is, connecting the “implementational” with the “representational” level [128]). Traditionally, behavioral studies of learning and memory have used test/pre-test comparisons of measures like latency or proportion correct as metrics of memory. While these metrics can effectively assess differences in memory strength between time points, they fail to provide insight into the underlying representations responsible for observed memory differences.

In Chapter 2, we found that disruption of BDNF receptor activity in the OB around the time of learning led to deficits in LTM, but not STM. We were unable to ascertain whether the deficit was due to disruptions to mechanisms that influence specificity and strength of odor representations. From the Selectivity Index (SI) measure, we saw that K252a-infused animals had poor LTM as well as lower certainty for the rewarded odor during LTM testing, but not STM testing. Although the SI is not a direct measure odor representations, the finding does prompt further investigation into the role of the BDNF pathway in representation specificity. In the current study, we directly investigate the role of the BDNF-TrkB pathway in the formation of odor representations using the olfactory generalization task (described in Chapter 1).

In this study, *bdnf* genetic variant mice were tested on the olfactory generalization task [183]. The variants were *bdnf* heterozygous knock-outs (*bgnf* +/-), [57] which have half the normal amount of BDNF, and the Val66Met knock-in (*bdnf* val/met and *bdnf* met/met) mice, which have near-normal levels of constitutive BDNF secretion but compromised activity-dependent secretion [36]. Thus, the combination of these models allows for the differentiation of the

unique contributions of the two modes of secretion to odor generalization. A separate group of wild-type mice were given OB-specific infusions of K252a prior to testing on the olfactory generalization task in order to ascertain whether observed effects were due to prolonged developmental deficits in the variants. Briefly, we found that Val66Met mice were able to form specific odor representations, whereas *bdnf* heterozygote mice were not. Wild-type mice that received K252a infusions did not differ from control animals in their formation specific odor representations, even though the infusion did influence their performance on a non-associative olfactory task.

3.3 Materials and Methods

3.3.1 Animals

A total of 30 genetic variant mice were used. They were heterozygous BDNF (BDNF+/-) and BDNF Val66Met knock-in (BDNFVal/Met and BDNFMet/Met) mice and were all maintained on an inbred C57BL/6 background. Behavioral experiments with the genetic variants were performed by Natalie Mandarion, Francis Lee, and Thomas Cleland. Additionally, a total of 19 adult male C57BL/6 mice (Charles River) were used. All experiments were performed on 8- to 10-week-old adult mice. Animal care was in accordance with Weill Medical College of Cornell University Institutional Animal Care and Use Committee and Food and Drug Administration standards. All procedures were performed under the auspices of a protocol approved by the Cornell University Institutional Animal Care and Use Committee (IACUC). Cornell University is accredited by

The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). The mice were maintained on a 12:12 hour reverse light/dark cycle.

3.3.2 Olfactory bulb cannulations

Wild-type C57BL/6 mice were anesthetized with gaseous 4% isoflurane (Henry Schein, Dublin, OH, USA) in pure oxygen and secured into a stereotaxic apparatus (Kopf Instruments, Tujuna, CA, USA). For the duration of the surgery, mice were kept under 1.5-2% isoflurane anesthesia through a nose cone. Guide cannulae (26-gauge; PlasticsOne, Roanoke, VA, USA) were inserted into both OB using the following coordinates with respect to bregma: AP +5.0mm, ML +/-0.75mm, DV 1.0mm. Two screws were drilled into the skull over the cerebellar formation to provide an anchor for the dental cement cap. Dental cement was used to secure the guide cannulae to these screws and to cover the incision area. Dummy plugs were placed into the guide cannulae to prevent blockage and infection. For two days after the surgeries, mice were fed softened food and given injections of an analgesia, ketoprofen (0.2mg/kg mouse), and saline. Animals recovered for at least a week before beginning behavioral training. Note that genetic variant mice were not cannulated because they did not receive infusions.

3.3.3 Infusions

Animals received OB-specific infusions of tyrosine kinase receptor inhibitor, K252a (Sigma-Aldrich K2015; 5% DMSO in saline) or a vehicle (saline with 5% DMSO). Drug or vehicle was delivered bilaterally into the OB at a volume of 2.0 μ L and 0.2 μ L/min (so the total time of the infusion was 10 minutes). Mice were not anesthetized during the infusions. Injectors were left inside cannulae for an additional 5 minutes to allow diffusion of the remaining liquid. Mice received infusions immediately before training on the olfactory generalization task and the spontaneous discrimination task.

3.3.4 Olfactory generalization task

Odor sets. Five odor sets were used, each composed of 6 odorants: the conditioned odorant (CS), a homologous series of sequentially similar odorants (S1-S4), and a structurally and perceptually dissimilar odorant (D). Each odorant was diluted in mineral oil to achieve a theoretical vapor-phase partial pressure of 0.1 Pa above the scented dishes [41].

Apparatus. Animals were tested in a clear Plexiglass cage (28 cm long \times 17 cm wide \times 12 cm high) with a removable black opaque center divider. Animals were placed into the resting chamber, at the beginning of each session. One trial included the lifting of the divider, animals entering the test chamber, and returning to the resting chamber.

Shaping. Animals underwent a behavioral shaping period prior to behavioral testing. Animals were brought to the experiment room and handled for 10 minutes per day for the first two days after recovery from surgeries. Next, animals were introduced to the behavioral apparatus. Two petri dishes (Pyrex, 60 mm diameter, 15 mm height) of scented sand (Quikrete; Atlanta, GA), limonene and mineral oil, were placed into the Plexiglass chamber without the center divider. These dishes were filled with 10-15 5-mg sucrose pellet reward (PJ Noyes Precision Pellets; TestDiet, Richmond, IN). Ten sugar pellets were mixed into the limonene-scented dish at various depths. Animals were placed into the test chamber for 10 minutes. They were allowed to freely explore the chamber and consume the sugar pellets.

On subsequent shaping days, animals were introduced to a shortened version of the final testing procedure. Again, two scented dishes were placed into the behavioral apparatus, including the center divider. A single sugar pellet was place on top of the limonene-scented sand. The animals were placed into the resting chamber. The center divider was lifted and animals were allowed to enter the test chamber and retrieve the sugar pellet. Animals were either ushered back into the resting chamber when they retrieved the sugar pellet or after 5 minutes elapsed. This was repeated for 12 trials. On a given trial, the dishes were randomly placed on the left or right using a random number generator. The whole procedure was repeated the next day. Crucially, the sugar pellet was buried progressively deeper with each trial. Shaping was considered complete when the mouse would reliably identify the reward-containing dish and retrieve deeply buried rewards, and dig in the odor-containing dish even in the absence of a reward (thus controlling for the possibility of the mouse directly detecting the reward).

Behavioral testing. We used a forced-choice olfactory generalization paradigm to measure the degree to which mice generalized a learned contingency (expectation of reward) from the conditioned odorant (CS) to each of the test odorants. Each mouse was trained over seven sequential conditioning trials in which it had a choice between a dish scented with CS (containing a 5 mg sucrose reward) and an unscented dish containing no reward. Subsequently, three unrewarded test trials (in which the mouse was offered a choice between a dish scented with one of the test odorants and an unscented dish) were performed in a pseudorandom, counterbalanced order; these test trials were alternated with two additional rewarded conditioning trials to prevent extinction of the association between the conditioned odorant and reward. Each six-odorant odor set was tested over two separate days to avoid satiation. During test trials, the total time spent digging in the dish containing the test odorant within the one-minute trial period was recorded with a stopwatch.

3.3.5 Spontaneous discrimination task

Odor sets. All odorants were diluted in mineral oil to give a theoretical vapor pressure of 0.01 Pa [41]. The odor sets used were the same as those previously published [13]. The key is that a given habituation odor is presented 4 times followed by single, counter-balanced presentations of 2 perceptually similar odors, S1 and S2, and 1 different odor, D. The odors are presented in teaballs, where 60uL of each odourant is pipette onto a piece of #1 Watman filter paper and placed into the teaballs. The teaballs are lowered into testing chambers at the start of each trial.

Shaping. Although no formal shaping is needed for animals to perform the spontaneous discrimination task, animals were given 2-3 days to acclimate to the testing apparatus. Specifically, animals were placed into a clear Plexiglass cage (28 cm long x 17 cm wide x 12 cm high) with a breathable lid. A teaball with Mineral Oil-scented filter paper inside was placed into the cage with the animal for 1 minute.

Behavioral testing. We used previously published methods [13] for the spontaneous discrimination task. In brief, animals were placed into the testing cage and given a series of odor exposures in teaballs. They were given one trial of mineral oil, followed by 4 presentations of a habituation odor, then the 3 test odors in pseudorandomized order. Each odor was presented for 50 seconds and a 5-minute inter-trial interval between each presentation. We used a stopwatch to record the investigation time for each odor. Animals were considered to be investigating the odor if they were actively sniffing within 1 cm of the teaball.

3.3.6 Data analysis

For both the olfactory generalization task and spontaneous discrimination task, we performed linear mixed effects analyses on the digging time and investigation time measures. Mixed effects models are similar to the well-known repeated measures analysis of variance (ANOVA) tests, however they are better suited for data sets with a hierarchical structure (e.g. differences in observations across time between multiple groups). The fixed effects for each analysis were Group (*bdnf* variant strain or K252a/vehicle) and Odor (CS, S1, S2, S3, S4, and D). Crucially, in all the analyses, we included random effects for individ-

ual mouse and odor sets nested within mouse. The random effects account for variance introduced by non-experimental factors, such as baseline odor preferences by individual mice. We used estimated marginal means to perform post-hoc pairwise comparisons on significant interactions from the full model. A Bonferroni correction for multiple comparisons was used for post-hoc pairwise comparisons.

All analyses were run using IBM SPSS 22.0.

3.4 Results

3.4.1 Olfactory generalization is unaffected in mice with Val66Met *bdnf* single-nucleotide polymorphism

First, we analyzed generalization gradients for the three SNP genotypes. We ran a linear mixed model with two fixed effects, Genotype (val/val, val/met, met/met) and Odor (CS, S1, S2, S3, S4, and D). Mouse and odor set nested within mouse were random effects in our model. We observed a significant main effect of Odor ($F(5, 338.939) = 23.038, p < .001$) and no significant interaction of Genotype and Odor ($F(10, 338.933) = .371, p = .959$), showing that the three genotypes did not differ in olfactory generalizations (Fig. 3.1).

Post-hoc tests, using the Bonferroni adjustment for multiple comparisons, confirmed that the variants did not differ in investigation time on any of the odors ($p > .05$ for all comparisons). And specifically, val/met and met/met mice did not differ from the wild-type strain, val/val ($p > .05$ for all comparisons).

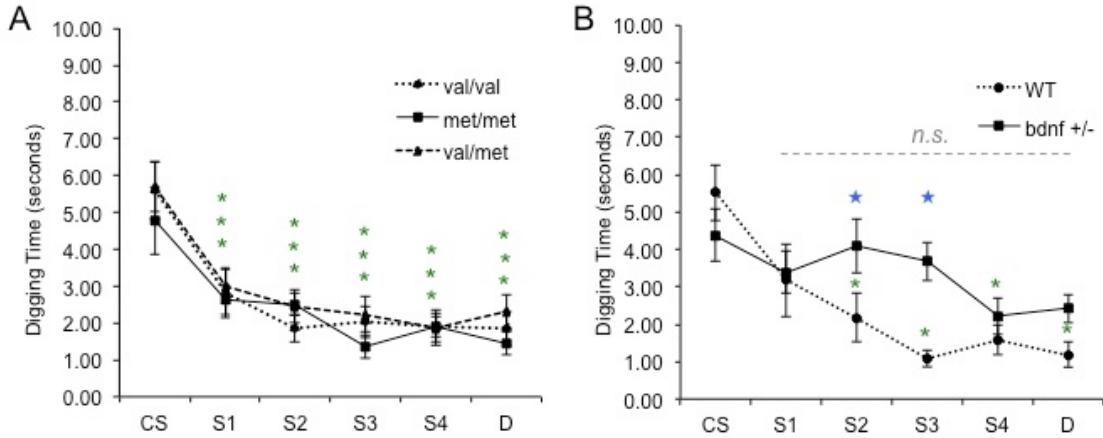


Figure 3.1: Olfactory generalization gradients for *bdnf* genetic variant mice are shown. **(A)** All variants of the Val66Met knock-in model show normal olfactory generalization with all three genotypes significantly differentiated the conditioned odor (CS) from all other test odorants ($p < .05$ in all cases). **(B)** *bdnf* heterozygous knockout animals showed compromised olfactory generalization ($p > .05$ for all). Wildtype mice significantly differentiated the conditioned odor (CS) from all other odors except S1 ($p = .084$ for S1, $p < .001$ for all other odors). Data generated by Natalie Mandairon, Francis Lee, and Thomas Cleland (unpublished data).

In addition, subsequent multiple comparisons confirmed that mice of all three genotypes significantly differentiated the conditioned odor (CS) from all other test odorants ($p < 0.05$ in all cases).

3.4.2 Heterozygous *bdnf* knockout mice show compromised olfactory generalization

Wild-type (WT) and *bdnf* knockout heterozygous were compared using a linear mixed model with two fixed effects, Genotype (WT, *bdnf* +/-) and Odor (CS, S1, S2, S3, S4, and D). Mouse and odor set nested within mouse were random ef-

fects in our model. We observed a significant interaction of Genotype and Odor ($F(5, 243.735) = 2.680, p = .022$), showing that the genotypes differed in olfactory generalizations. We also observed main effects of both Genotype ($F(1, 49.353) = 4.495, p = .039$) and Odor ($F(5, 243.735) = 8.589, p < .001$).

Post-hoc comparisons testing using the Bonferroni correction demonstrated that wildtype mice significantly differentiated the conditioned odor (CS) from all other odors except S1 ($p = .084$ for S1, $p < .001$ for all other odors), whereas digging times in all the odors were not significantly different from CS ($p > .05$ for all) for the *bdnf* heterozygotes (Fig. 3.1).

3.4.3 Olfactory generalization is not affected by acute neurotrophin receptor blockade in the olfactory bulb

To analyze the effects of OB-specific infusions of K252a on olfactory generalization, we used a linear mixed model with two fixed effects, Drug (K252a or Vehicle) and Odor (CS, S1, S2, S3, S4, and D). Mouse and odor set nested within mouse were random effects in our model. We observed a significant main effect Odor ($F(5, 351.635) = 7.823, p < .001$), but no interaction of Drug and Odor ($F(5, 351.635) = .571, p = .722$) suggesting that the groups did not differ in their olfactory generalization.

Indeed, post-hoc comparisons with the Bonferroni correction for multiple comparisons showed that digging time did not differ between K252a and Vehicle groups for any of the odors ($p > .05$ for all odors). Additional comparisons showed that animals in the Vehicle significantly differentiated the conditioned

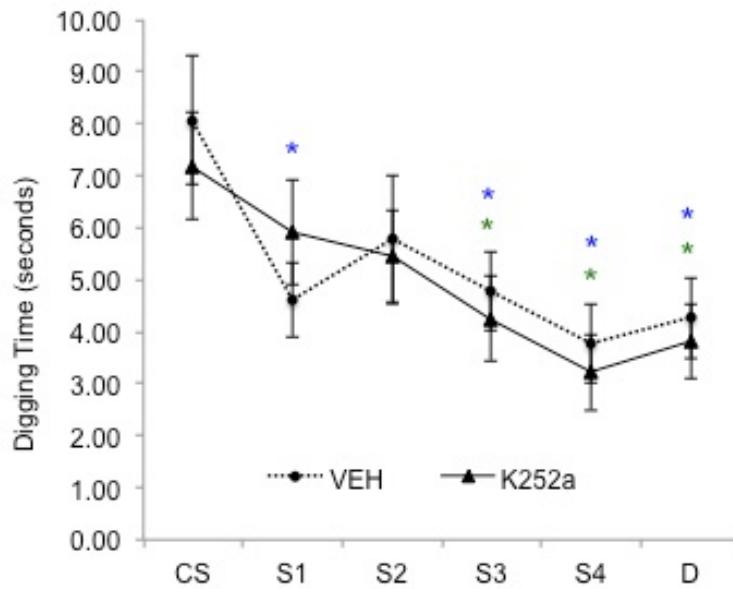


Figure 3.2: Olfactory generalization gradients following OB K252a infusions. Gradients for drug and vehicle animals did not differ as digging times were not different for any of the odors ($p > .05$ for all odors). Vehicle significantly differentiated the conditioned odor (CS) from all other odors except S2 ($p = .649$ for S2, $p < .05$ for all other odors). For K252a animals, digging times in S3, S4, and D was significantly different from CS ($p < .05$ for all).

odor (CS) from all other odors except S2 ($p = .649$ for S2, $p < .05$ for all other odors). Likewise, digging times for K252a animals in S3, S4, and D was significantly different from CS ($p < .05$ for all). Fig. 3.2 shows the generalization gradients for these animals.

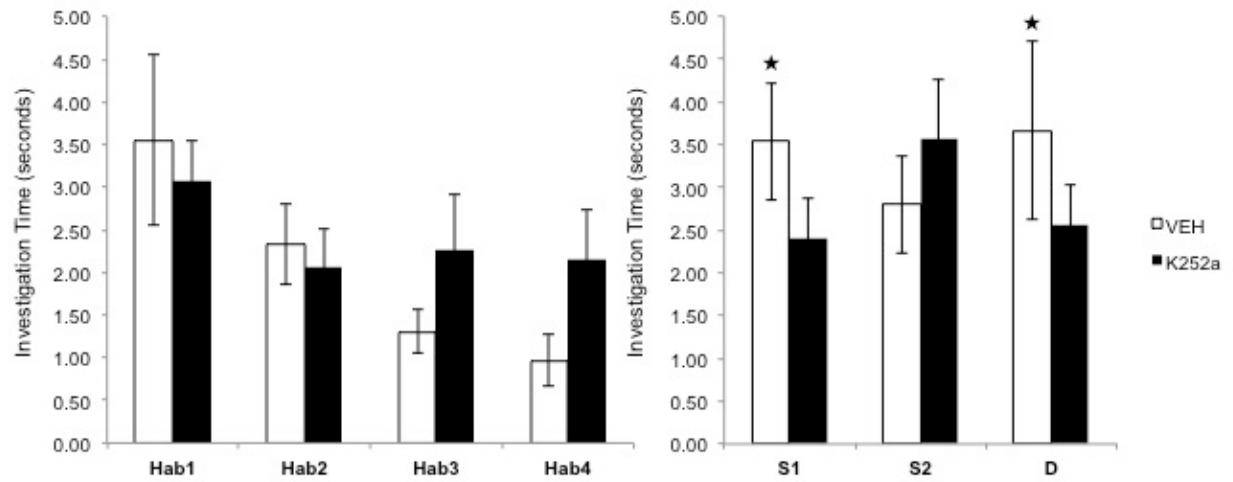


Figure 3.3: We observed no significant differences in the pattern of habituation between the K252a and Vehicle groups ($F(3, 113.707) = 1.626, p = .187$). Vehicle animals expectedly dishabituated, or increased their investigation times, for S1 and D ($p < .05$ for both) compared to Hab4.

3.4.4 Effects of acute neurotrophin receptor blockade in the olfactory bulb on habituation and spontaneous discrimination

As a drug-efficacy control, we tested K252a-infused mice on a non-associative habituation/spontaneous discrimination task. We used a linear mixed model to analyze the data. The model included two fixed effects, Drug (K252a or Vehicle) and Odor Trial (either Hab1, Hab2, Hab3, and Hab4 or Hab4, S1, S2, and D). As before, we included mouse and odor set nested within mouse as random effects. Because the habituation phase (the 4 consecutive presentations of the habituation odors) and the spontaneous discrimination phases (presentations of S1, S2, and D relative to the fourth Hab presentation) can be thought of as

testing two different types of behavior, we used two separate analyses for these phases. The first model included trials Hab1, Hab2, Hab3, Hab4 and the second included Hab4, S1, S2, and D.

The full model for the habituation phase showed a significant main effect of Odor Trial ($F(3, 113.707) = 5.535, p = .001$), but no significant main effect of Drug ($F(1, 123.078) = 1.953, p = .165$), and no significant interaction of Drug and Odor Trial ($F(3, 113.707) = 1.626, p = .187$). If the Drug and Vehicle groups did differ on their patterns of habituation, as they appear to visually (Fig. 3.3), we would have expected a significant 2-way interaction.

We ran a separate linear mixed effects model for the spontaneous discrimination phase and found a significant 2-way interaction of Drug and Odor Trial ($F(3, 114.706) = 2.954, p = .036$) and a significant main effect of Odor Trial ($F(3, 114.706) = 4.653, p = .004$). Post-hoc comparisons showed that the drug and vehicle groups did not differ from each other during Hab4 ($p = .064$), S1 ($p = .204$), S2 ($p = .195$), or D ($p = .210$). For the Vehicle group, we found that S1 and D were both significantly higher than Hab4 ($p < .05$ for both; $p = .064$ for S2). For the drug group, none of the test trials were significantly different from Hab4 ($p > .05$ for S1, S2, and D).

Together, the statistical analyses do not support the significance of the observed differences in the pattern of habituation between the drug and vehicle (Fig. 3.3). On the basis of the significant main effect of Odor Trial, I observed in subsequent pairwise comparisons (with the Bonferroni correction) that while investigation time between Hab1 and Hab3 ($p = .006$), and Hab1 and Hab4 ($p = .001$) were significantly different for the Vehicle group, no trials were different for the K252a-infused group ($p > .05$). In light of the non-significant interaction

between Drug and Odor Trial in the omnibus F test, these pairwise comparisons cannot be used to establish a significant difference in habituation patterns between the drug and vehicle group. However, the combined evidence of these findings and the strong observed differences in Fig. 3.3 suggests that the effect of K252a-infusions on habituation warrants further investigation.

3.4.5 Effect of neurotrophin receptor blockade on odor detection

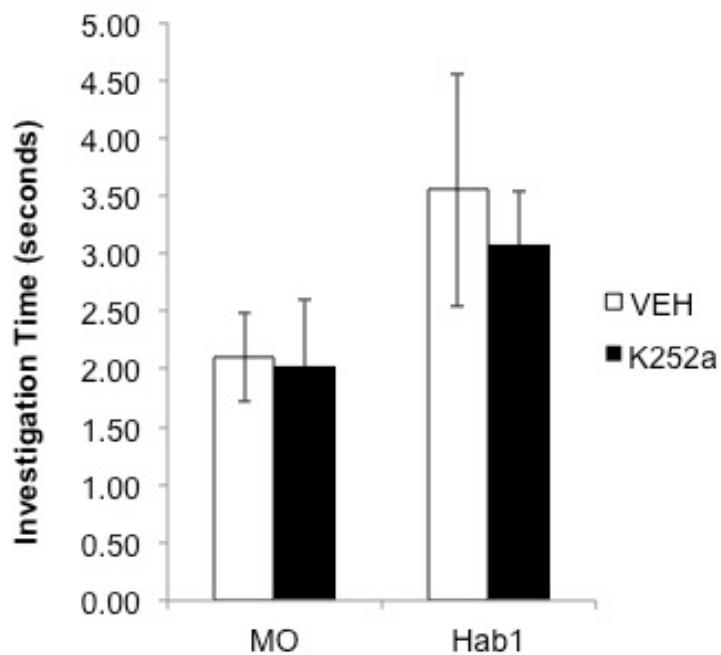


Figure 3.4: Linear mixed effects analysis with Drug and Odor Trial (Mouse and odor set nested within mouse as random effects.) resulted in a significant main effect of Odor Trial $F(1, 48.868) = 6.341, p = .015$, and no other significant effects.

Finally, we compared investigation time of the initial mineral oil presentation with the investigation time of the first presentation of the habituation odor

as a means of testing odor detection. Again, we ran a linear mixed effects analysis with two fixed effects, Drug (K252a or Vehicle) and Odor Trial (MO and Hab1). Like before, we included mouse and odor set nested within mouse as random effects. We found only a significant main effect of Odor Trial ($F(1, 48.868) = 6.341, p = .015$) suggesting that, on average, animals increased their investigation of Hab1 (Fig. 3.4).

3.5 Chapter 3 Discussion

Our goal in this set of experiments was to examine the role of the BDNF-TrkB pathway in the formation of OB-dependent representations of reward-associated odors. We found that *bdnf* heterozygous mice with global, developmental deficits in BDNF showed deficits in the formation of odor representations. However, Val66Met animals (with compromised activity-dependent BDNF secretion only) did not differ from wild-type mice in their olfactory generalization behavior. Importantly, we also found that animals who received OB-specific infusions of the neurotrophin receptor blocker prior to conditioning did not differ from control animals in their olfactory generalizations. Together, the finding suggests that a baseline level of constitutive BDNF is needed in the developing brain for appropriate representational learning in adulthood. Additionally, neurotrophin activity in wild-type adult mice is not needed during acquisition for the formation of appropriate odor representations.

In the previous chapter, we saw that neurotrophin blockade in OB led to LTM, but not STM, deficits in an odor-reward association task. Although we did not observe differences in learning rate for the odor-reward association us-

ing the proportion correct measure, it is still conceivable that the observed LTM deficit resulted from disruptions to mechanisms involved in odor representation formation. In the present study, we found that K252a-infusions did not disrupt the formation of odor representations. Together, the findings from the 2 chapters suggest that the BDNF-TrkB pathways in the OB is truly not needed during information acquisition. Rather, the pathway may be specifically important during the post-learning period for the consolidation of odor representations. This idea is corroborated by studies looking at the cellular effects of BDNF. In addition to its necessity for the induction of LTP [151], [120] also found that bath application of BDNF was sufficient to induce L-LTP from E-LTP. The finding suggests that BDNF plays a critical role in mechanisms underlying LTM consolidation, but not acquisition. However, we still do not know what that means in terms of the shape of representations. An important future study will be to examine, using the olfactory generalization task, long-term odor representations after neurotrophin receptor blockade prior to learning.

We additionally observed that K252a-infused animals may fail to habituate to repeated presentations of an odor, although the pattern is not statistically confirmed. This test was intended to be a positive control for K252a efficacy as well as a demonstration that BDNF receptor activity in the OB plays a role in the online processing of odors. Previously, Bath and colleagues [13] studied habituation in a set of BDNF genetic variants. The authors found that both *bndf* heterozygote knockouts and Val66Met knock-in mice show normal odor habituation behavior. However, they found additionally that *trkb* heterozygote knockout animals do not habituate to repeated presentations of an odor and show an “atypical” habituation pattern, a result that we would have expected from our experiment. Together, it may suggest that manipulation of the *receptor*,

rather than the ligand, is most influential for odor habituation.

McNamara and colleagues [136] found that NDMA receptor antagonists in the OB prevented habituation in an identical paradigm, and that this lack of behavioral habituation was associated with persistent mitral cell (MC) firing [34] to repeated odor exposure. This type of physiological activity could occur due to decreased odor response in the olfactory sensory neurons (OSNs) or due to decreased inhibitory input onto the MCs. This latter activity likely explains our observed results. A strong piece of evidence to support this idea comes from [75]. The authors found that reciprocal GABA release from granule cells (GCs) into the GC-MC synapse is directly regulated by Ca^{2+} influx through NMDARs on GCs, suggesting that NDMAR blockade would prevent GC inhibition. Other studies additionally show that NMDA receptor activity is importantly involved in GC inhibition of MCs [191, 170]. Given the fact that TrkB receptors are expressed in similar neuronal populations as NMDA receptors in the OB [18], I suggest that TrkB inhibition prevents habituation through the decrease of MC inhibition. In addition, a study [150] that used an arguably stronger odor exposure protocol, 30-seconds of electrical stimulation to the dorsal OB, researchers found that glomerular response was significantly decreased for 4 minutes after the odor stimulation and had returned to base-line by 6 minutes. In the current study, we used 50-second odor presentations with 5-minute inter-trial intervals, which should allow for considerable neuronal refractory period. Thus, habituation at the longer timescale used in our experiment is not explained by bottom-up neuronal adaptation.

The finding that NMDA receptor inhibition in the OB prevents odor habituation also shows that canonical LTM mechanisms can and do participate in on-

line odor processing. One proposal for the purpose of this dual involvement is known as the “synaptic tag hypothesis” [66] and aims to describe how synaptic specificity is achieved within neuronal ensembles. During learning, “tags” are set across the synapses which were activated by that learning event. Later, these tags sequester plasticity-related proteins (PRPs) selectively at the previously potentiated synapses and the PRPs launch molecular pathways for long-term consolidation. Thus, a synaptic tag importantly participates in online information processing (or acquisition) and has important later effects on consolidation processes. This idea that memory consolidation depends on coordinated, multiphasic activity has only recently been directly tested in a behavioural paradigm [50]. The study found that exposure to a novel environment 1-2 hours before or one hour after fear extinction training enhanced extinction and this enhancement was attenuated by infusion of protein synthesis inhibitors into the HPC. The authors argue that novelty exposure induces the synthesis of PRPs and extinction training sets synaptic tags at the synapses involved. These tags incidentally capture the previously released PRPs and, thus, the enhancement can occur as long as the PRPs are active within the lifetime of the synaptic tag.

The BDNF-TrkB pathway is a candidate system for a synaptic tag because its involvement in behavioral memory paradigms so closely matches its observed molecular timeline. For example, using an inhibitory avoidance (IA) paradigm, [120] manipulated the strength of a shock so that a weak shock induced STM, but not LTM, for the task. They found that blockade of TrkB activity did not reduce the expression of the one-hour STM. In a second set of experiments, animals were exposed to a novel environment one hour prior to receiving the weak IA training. In these animals, memory for the task persisted beyond 24 hours where it otherwise would not have. The blockade of a transient, phosphory-

lated TrkB peak thirty minutes after E-LTP induction prevented the induction of L-LTP. Together the results show that, at least *in vitro*, TrkB receptor activity immediately after activity-induced plasticity is needed for longer lasting forms of plasticity. Another strong piece of evidence for the idea of the BDNF-TrkB pathway as a synaptic tag comes from its matching behavioral and molecular timelines. [66] found that “synaptic tags” decayed within three hours of the initial tetanus, after which further stimulation did not induce L-LTP. This timing matches well with behavioral work from [7] showing that BDNF is needed for memory retention up to three hours following IA training, but not needed six hours later. Future studies that pair behavioral paradigms with pharmacological or genetic TrkB blockade could more directly explore the functional role of the TrkB receptor as a “synaptic tag”.

In Chapter 2, we showed that the BDNF-TrkB pathway is needed around the time of multi-trial learning for LTM, but not STM. In the current chapter, we used a behavioral measure of “odor representations” and showed that while BDNF is needed during development for normal representational learning in adulthood, acute activity of the BDNF-TrkB pathway in the OB did not affect the acquisition of an odor representation. The finding suggests that the BDNF-TrkB pathway in the OB influences LTM through post-learning consolidation processes, not during learning. We also observed a trend that blockade of neurotrophin receptors in the OB prevented habituation to repeated odor presentations, suggesting that BDNF’s receptor, TrkB, plays a role in online odor processing, as well as, its putative role in LTM consolidation. We discussed how this pattern of activity is consistent with the role of TrkB as a synaptic tag. Future studies, however, will need to look at this more directly in the OB for multi-trial olfactory memories.

CHAPTER 4

**TIMECOURSE OF PLASTICITY-RELATED ACTIVITY FOLLOWING
ASSOCIATIVE LEARNING**

4.1 Abstract

Previous studies have contributed to a strong understanding of the role of many molecular mechanisms involved in LTM, including describing the effects of those mechanisms on longer-term structural changes to neuron ensembles involved in LTM consolidation. However, this research has also suggested that LTM depends as much on the temporal specificity of these mechanisms as their downstream effects. In addition, it is yet unclear from this work how the timing of these mechanisms is coordinated across the multiple brain regions involved in learning. In the present study, we take a first step toward characterizing the timecourse of several molecular mechanisms across multiple brain regions. We train mice on an associative odor learning task for 1, 2, 4, or 6 days. We collected the OB, striatum, hippocampus, cortex, and cerebellum from the mice on each day prior to training, immediately after training, or 15, 30, or 60 minutes after training. We then used high-throughput RT-PCR to analyze mRNA levels for several plasticity-related proteins (PRPs), including *bdnf*, intracellular signaling cascades, *erk1* and *erk2*, transcription factor, *creb1*, and immediate early genes, *arc*, *fos*, and *erg1*. We found that learning-responsive transcription differed between genes, and that PRP timecourses differed as a function of brain region. Future studies could use this “spatiotemporal” map to discover the functional consequences of these patterns.

4.2 Introduction

Molecular mechanisms (including intracellular cascades, molecular signaling, neuromodulatory influences, activity-dependent protein synthesis, and epigenetic modifications) have been shown to underlie LTM consolidation and persistence through their role in long-term structural changes (such as long-term potentiation or other synaptic weight changes, alterations to neuronal morphology such as dendritic branching, changes to terminal shapes or numbers, and even ancillary modifications such as effects on glia or cell adhesion to the extracellular matrix, and changes to neuron number via adult neurogenesis or selective apoptosis).

A great deal of work, particularly those using the inhibitory avoidance (IA) paradigm, have contributed to our knowledge of the functional roles of these molecular mechanisms. IA conditioning leads to a rapid elevation of several intracellular signalling pathways in the hippocampus (HPC), including CaMKII, cAMP, the LTM-associated transcription factor CREB [60]. Learning promotes the trafficking of receptors in the plasma membrane, including ionotropic glutamate receptors [163, 47], and increases the transcription of other plasticity-related mechanisms, like BDNF [35]. These mechanisms and their downstream structural effects were extensively reviewed in Chapter 1 and shown in Fig. 1.2.

Of particular relevance to our present discussion is the fact that work examining the activity of these mechanisms suggests that LTM consolidation relies as much on the temporal specificity of the mechanisms as it does on their downstream effects. For example, blocking CaMKII activity immediately after IA training substantially reduced animals' fear responses when measured

24 hours later (i.e., LTM). However, blocking CaMKII activity 30 minutes after IA resulted in a weaker LTM deficit, and blockade 2-4 hours after IA had no effect on LTM at all (Figure 1.2) [192]. Studies that directly measured CaMKII levels revealed that CaMKII activity increased immediately after IA training, and remained high when tested 30 minutes later, but had returned to baseline when tested two hours after conditioning [20]. These findings indicate that CaMKII plays a crucial role early in the memory induction process, and that its functional role in LTM formation is confined to a specific period following learning. Similar results for other mechanisms, including PKA [22, 21, 20, 24, 31, 83, 87, 192], NMDA and AMPA [23, 81, 86], and BDNF [15], are shown in Figure 1.2.

In addition to the importance of understanding timecourse, limited studies have also suggested that timecourses for the mechanisms differ across brain regions in meaningful ways. For example, [85] gave infusions of either NDMA or AMPA receptor antagonists into the amygdala, HPC, and entorhinal cortex either 0, 90, 180, and 360 minutes after IA training. Amongst other results, the authors found that 24-hour memory was significantly attenuated in animals that received amygdala and HPC infusions of the NMDA receptor antagonist, AP5, immediately after training. Twenty-four hour memory was unimpaired in animals that received this infusion into the entorhinal cortex. However, 90- or 180-minute infusions into the entorhinal cortex did have amnesiac effects. The finding implies that NMDA receptor activity in these areas have functionally different time courses.

Studies of other plasticity-related mechanisms in multi-trial, appetitive learning paradigms, such as a radial arm maze task, have shown that time-

courses can differ with one-trial learning. In the HPC, BDNF levels were found to increase immediately after IA training. Infusions of function-blocking BDNF antisense oligonucleotides into the HPC just prior to training blocked LTM consolidation, while sparing STM, indicating that BDNF activity immediately following learning sets the stage for eventual LTM consolidation [5, 15]. Similarly, BDNF mRNA levels in the HPC increased significantly after conditioning on an appetitive radial arm maze task – but only on the eighth day of training [142]. Additionally, while BDNF mRNA levels increased 15 minutes after the last training trial in the HPC, no increase was observed during this time in the frontal cortex [143]. These findings suggest that, first, similar molecular mechanisms are involved in multi-trial appetitive learning as in single-trial, fear-based learning, but, crucially, the timecourse after learning differs dramatically (that is, on the order of days, as opposed to minutes or hours). Second, they corroborate results that, indeed, the timecourse of plasticity-related activity differs between different brain regions.

In addition to relying primarily on one-trial learning, a weakness of previous studies is that very few have examined the mechanisms across regions for the same learning event. In the present study, we take a first step toward characterizing the timecourse of several molecular mechanisms across multiple brain regions during the same multi-trial learning event. We train mice on an associative odor learning task for 1, 2, 4, or 6 days. We collected the OB, striatum, hippocampus, prefrontal cortex, and cerebellum from the mice on each day prior to training, immediately after training, or 15, 30, or 60 minutes after training. We then used high-throughput RT-PCR to analyze mRNA levels for several plasticity-related proteins (PRPs), including *bdnf* (and intracellular signaling cascades, *erk1* and *erk2*), *arc*, *fos*, *erg1*, and *creb1* in these tissues.

We found that learning-responsive transcription differed between genes, and that PRP timecourses differed between brain regions. Future studies could use this “spatiotemporal” map to discover the functional consequences of these patterns.

4.3 Materials and Methods

4.3.1 Animals

A total of 60 adult male CD-1 mice (Charles River) were used in this experiment. The mice were 8 weeks old at the beginning of the experiment. All procedures were performed under the auspices of a protocol approved by the Cornell University Institutional Animal Care and Use Committee (IACUC). Cornell University is accredited by The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).

The mice were kept on a 12:12 hour reverse light/dark cycle and allowed free access to water at all times. They were kept on a food-restriction schedule designed to keep them around 90% of their free feeding weight for the duration of the behavioral experiments. This food restriction schedule began 3 days before the beginning of behavioral tasks.

4.3.2 Behavioral Training

Apparatus.

Animals were tested in a clear Plexiglass cage (28 cm long x 17 cm wide x 12 cm high) with a removable black opaque center divider. Animals were placed into the resting chamber, at the beginning of each session. One trial included the lifting of the divider, animals entering the test chamber, and returning to the resting chamber.

Odor sets.

For shaping, we used (+/-)limonene (Sigma-Aldrich, St. Louis, MO, USA) as the rewarded odor and mineral oil as the unrewarded odor. For Training, we used butanoic acid and pentanoic acid. All odorants were diluted in mineral oil so as to emit a theoretical steady-state vapor phase partial pressure of 0.1 Pa [41]. This lower odor concentration was chosen so that animals would not reach asymptotic performance after the first day of training. The rewarded odor was counterbalanced between the mice to avoid odor preference biases.

Shaping.

Animals underwent a ten-day behavioral shaping period prior to Training with the test odors. The shaping procedures followed exactly those described in Chapter 2. The crucial idea in the present experiment is that all mice for all groups underwent the same amount of shaping prior to Training.

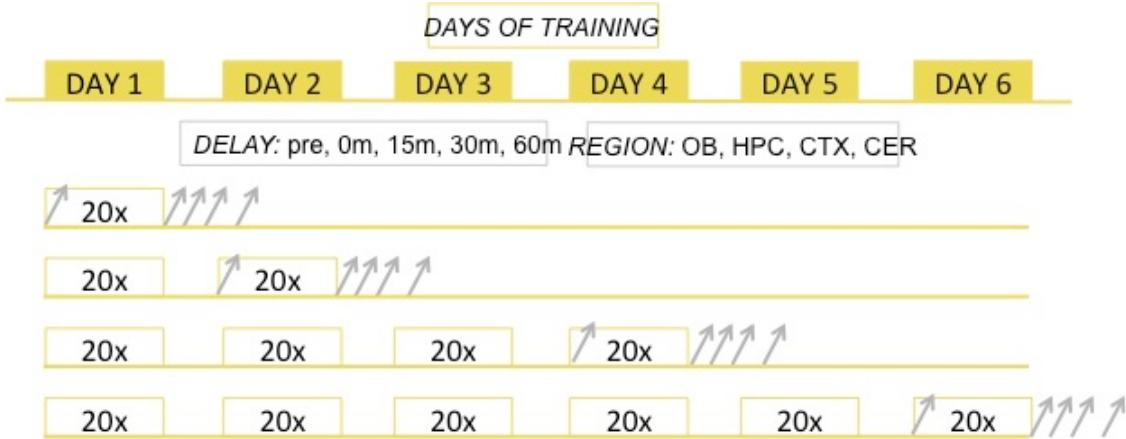


Figure 4.1: Associative learning protocol used to obtain mRNA data. Animals were given 20 trials (“20x”) of the odor-reward task across either 1, 2, 4, or 6 days. Grey arrows represent the “DELAY”s when brain tissue was harvested (immediately prior to behavioral training, immediately after, 15 minutes after, 30 minutes after, or 60 minutes after their final training trial. At those delays, the “REGION”s removed were the olfactory bulb (OB), the striatum (STR), the hippocampus (HPC), the cerebellum (CER), and roughly the prefrontal cortex (CTX).

Training.

The mice were divided into four Training groups. The groups received either one, two, four, or six days of training (Fig. 4.1). At the beginning of each day of training, the animals were placed into the resting chamber. Two dishes of sand scented with butanoic acid and pentanoic acid were placed in the test chamber. A sugar pellet was buried fully in the rewarded odor dish. If animals dug in the rewarded odor first, they were allowed to retrieve the sugar reward, ushered back into the resting chamber and the next trial began immediately. If the animal dug in the unrewarded odor first, they were ushered back into the resting chamber and the next trial began immediately. These trials lasted up to one minute. One day of Training included 20 of these trials.

4.3.3 Tissue Collection

Animals were sacrificed by rapid decapitation at various time points (Fig. 4.1) during the training day: immediately prior to their final day of behavioral training, immediately after, 15 minutes after, 30 minutes after, or 60 minutes after their final training trial. For the 15-, 30-, and 60-minute delays, animals were placed back into their home cages prior to sacrifice. After decapitation, brains were immediately removed and bilateral dissections were performed to remove the olfactory bulb (OB), the striatum (STR), the hippocampus (HPC), the cerebellum (CER), and roughly the cortex (CTX). Tissue was flash-frozen on dry ice.

This process was done on Training Days 1, 2, 4, and 6, with 1-3 biological replicates per time delay, such that the total number of tissue samples was 300.

4.3.4 Measuring mRNA levels by real-time, reverse transcriptase PCR

RNA extraction was performed by Michelle Tong and Madhura Raghavan. cDNA synthesis and qPCR reactions were performed by research collaborators Madhura Raghavan and Jeffrey Pleiss.

Total RNA extraction and isolation

A quarter of the homogenized tissue samples in Trizol were taken (500 µl) and laid out into four 96-well plates. The samples were grouped in such a way that two biological replicates of all time points for a particular tissue were present in

a single plate to enable within tissue comparisons. Additional biological replicates were grouped together on another plate to enable comparison between all the Day and Delay points. We added 6 control samples to all the plates in order to control for any technical variation arising from the handling of the plates. These were frozen at -20°C. On the day of RNA extraction, the plates were thawed at room temperature for an hour. (100 µl) of Chloroform was added to each samples, vortexed and set on bench top for 5 minutes until two phases became visible in Trizol. The plates were then spun at 4000 G at 4°C for 20 minutes. Using robotics, around 180 µl of the aqueous phase was transferred into a new 96-well plate. 1 ml of binding buffer (2M Guanidine Hydrochloride, 75% Isopropanol) was added to the aqueous phase and RNA was extracted using a 96-well glass fiber binding plate (NUNC 278010) with two successive washes with wash buffer (80% Ethanol, 10mM Tris pH 8) followed by a dry spin. The RNA was then eluted in 100 µl of water. The RNA yield was highest for cortex samples (~13 µg) and lowest for striatum samples (~1 µg).

cDNA synthesis

We used 80 µl of the total unit100µl of purified RNA for cDNA synthesis. Each sample of cDNA synthesis contained a total volume of 160 µl and which contained 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM each dNTP, 40 µg dN9 primer, and M-MLV RT. Reactions were incubated overnight at 42°C. On average, the cDNA yield was around 25%, with the CTX samples yielding ~3 µg cDNA and STR samples yielding ~300 ng cDNA. The cDNA samples were then diluted 2.5 fold to 400 µl with an effective concentration between ~7.5 ng/µl (STR) and ~7.5 ng/µl (CTX). Except for plate 2, the

cDNA was used without any further repurification. For technical reasons, Plate 2 samples were repurified by processing through a 96-well glass fiber binding plate (NUNC 278010) with 7 volumes of cDNA binding buffer (5M Guanidine Hydrochloride, 30% Isopropanol, 90mM KOH, 150mM Acetic acid) with two successive washes with wash buffer (80% Ethanol, 10mM Tris pH 8) followed by a dry spin. cDNA from this plate was then eluted in 50 µl of water twice and diluted to a total volume of 200 µl. This is effectively a 2-fold dilution compared to the cDNA on the other three plates (~3.75 ng/µl for cortical samples and ~0.375 ng/µl for samples of striatum).

QPCR and analysis

The QPCR reactions were performed in a reaction volume of 10 l, containing 5 µl of template (~1.875 ng to ~37.5 ng based on the cDNA estimates above), 10 mM Tris-HCl (pH 8.5), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each dNTP, 0.25x SYBR Green, 5% DMSO, Taq DNA polymerase, and 250 nM forward and reverse primers. The primer sequences for each targeted gene is shown in Table 4.1. Standard curves were generated consisting of 4-fold serial dilutions of cortex cDNA and covering a range of 1.6 × 10⁵ molecules. Each primer pair was well-behaved, showing an amplification efficiency of between 85% and 99%. Three technical replicates were measured for each biologically independent sample, generating up to nine independent measurements for each sample.

For each technical measurement, an amount value (arbitrary unit) of each tissue was calculated using the standard curves and averages calculated for each gene. We used an existing R package to determine which of the 3 housekeeping genes (β -Actin, GAPDH, or PGK1) we used was best for normalization. In these

analyses, we used β -Actin to normalize each sample. Analyses were then done using this normalized “amount” measure.

4.3.5 Data analysis.

For the behavioral data, we used analyses similar to Chapter 2. In brief, we averaged every 5 trials to create four Trial Blocks (TBs) on each day. Again, we performed a logit transformation prior to statistical analysis. We then used a linear mixed effects analysis on the transformed measures. Specific details of the model are reported with Results below.

For the RT-PCR data, we were interested in changes to mRNA transcript levels for each gene after multi-trial learning within each brain region, for each day of training. We used the amount, normalized to β -Actin levels, as the measure of mRNA transcript levels. We then used a linear mixed effects model with three fixed effects: Delay (immediately before training, “pre”, immediately after training, “0”, 15, 30, and 60 minutes after training), Region (cerebellum, cortex, olfactory bulb, hippocampus, striatum), and Day (1, 2, 4, and 6). Mouse and Region nested within Mouse were included as random effects. We ran a separate model for each gene of interest. We used estimated marginal means with a Bonferroni correction to perform pairwise comparisons on significant interactions from the full model.

All analyses were performed using IBM SPSS 23.0.

Table 4.1: Primer pairs used for qPCR analysis

Primers	Sequences
FWD_ACTB	CTG GCC GGG ACC TGA CAG ACT ACC
RC_ACTB	TCT TTG ATG TCA CGC ACG ATT TCC CT
FWD_ARC	CGC AGA AGC AGG GTG AAC CAC TCG
RC_ARC	GCA GAA AGC GCT TGA GTT TGG GCT G
FWD_BDNF	AGA AAG TCC CGG TAT CCA AAG GCC
RC_BDNF	ATT GGG TAG TTC GGC ATT GCG AGT
FWD_CREB1	AGT GCT TGA AAA CCA AAA CAA AAC
RC_CREB1	ATC TGA TTT GTG GCA GTA AAG GTC
FWD_EGR1	GGC CAA GGC CGA GAT GCA ATT GAT GT
RC_EGR1	AGC CCC GTT GCT CAG CAG CAT CAT CT
FWD_ERK1(MAPK3)	ACC TAC TGT CAG CGC ACG CTG AGG
RC_ERK1(MAPK3)	ACA TTC TCA TGG CGG AAT CGC AGC
FWD_ERK2(MAPK1)	GTC CAT TGA TAT TTG GTC TGT GGG CT
RC_ERK2(MAPK1)	TCA GCT GGT CAA GGT AAT GCT TTC CT
FWD_FOS	CCC CAT CCT TAC GGA CTC CCC ACC C
RC_FOS	CGC TCT GCC TCC TGA CAC GGT CTT CA
FWD_GAPDH	AGC CAA AAG GGT CAT CAT CTC CGC
RC_GAPDH	GGT GCA GGA TGC ATT GCT GAC AAT C
FWD_PGK1	GCC AAG TCC GTT GTC CTT ATG AGC C
RC_PGK1	CCA GCA GAG ATT TGA GTT CAG CAG CA

4.4 Results

4.4.1 Animals learn odor-reward association and show asymptotic performance by the third day of training

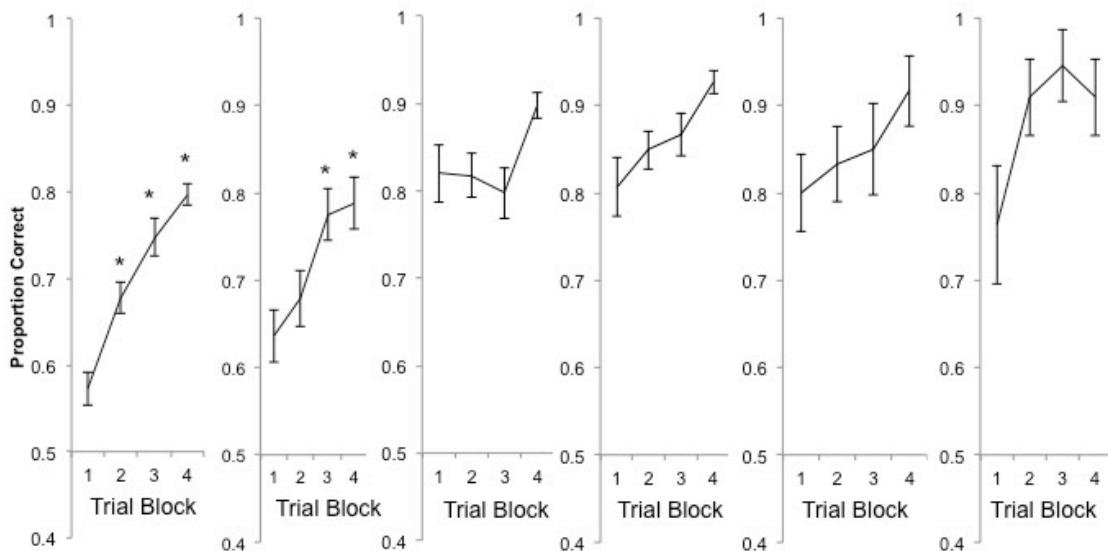


Figure 4.2: Animals show greatest learning during the first and second day of training. Stars represent Trial Blocks (TBs) which are significantly higher than the first TB of each day ($p < .05$). Performance asymptotes on the third day of training.

First, we analyzed the learning curves for all the days of training to assess patterns of learning. We ran a linear mixed model with three fixed effects, Day (1, 2, 4, or 6), Trial Block (TB1-TB4 within each day), and Odor (Butanoic Acid or Pentanoic Acid). Mouse was included as a random effect in our model. In order to test the patterns of learning across the days, we look at the interaction of Day and Trial Block, as well as their main effects. In order to test whether or not animals favored one odor over the other, we tested for a main effect of

Odor. We observed a significant main effect of TB ($F(3, 622.426) = 10.435, p <.001$), a significant main effect of Day ($F(3, 622.114) = 11.7835, p <.001$) and no significant interaction of Day and TB ($F(3, 622.426) = 1.034, p = .418$). Finally, we found no significant main effect of Odor ($F(1, 42.225) = .575, p= .452$) showing that performance was the same for animals regardless of which odor was used as the rewarded.

Post-hoc tests, using the Bonferroni adjustment for multiple comparisons, showed that on Day 1 of training, TB2, TB3, and TB4 were significantly higher than TB1 ($p <.05$ for all comparisons). On Day 2 of training, TB3 and TB4 were significantly higher than TB1 ($p <.05$ for all comparisons). For Day 3, Day 4, and Day 5, none of the trial blocks were significantly higher than TB1 for that day ($p >.05$ for all comparisons) suggesting that learning occurred during Day 1 and Day 2 and had saturated by Day 3 of training (Figure 4.2).

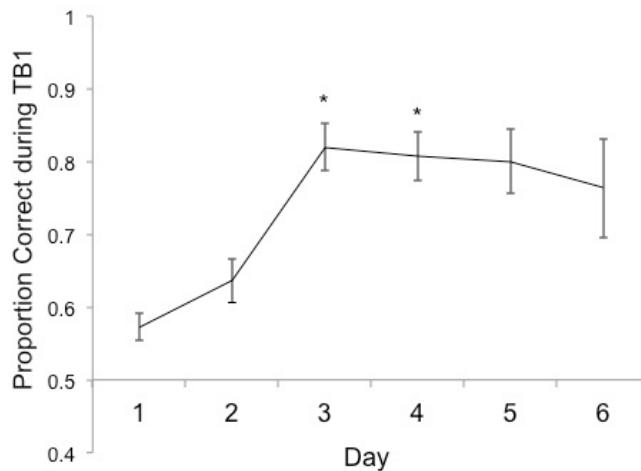


Figure 4.3: TB1 on Day 3 and Day 4 were significantly higher than TB1 on Day 1 ($p <.001$ for all comparisons). TB1 on Day 5 and Day 6 were not significantly higher than TB1 on Day 1 ($p >.05$ for both comparisons).

When we compared the first Trial Block of each day, specifically, we like-

wise saw that TB1 on Day 3 and Day 4 were significantly higher than TB1 on Day 1 ($p < .001$ for all comparisons). TB1 on Day 5 and Day 6 were not significantly higher than TB1 on Day 1 ($p > .05$ for both comparisons). We suspect this is because the number of animals were lower for these two days compared to the other days which led to higher variance in these groups. However, we observed that performance remained plateaued around 0.8 on these days. Taken together with the observations above that TB1 did not significantly differ from the other trial blocks on Day 5 and Day 6, we suggest that animals demonstrated behavioral performance which asymptotes by Day 3 of training (Figure 4.3).

4.4.2 Brain-derived neurotrophic factor mRNA levels differ between brain regions

For BDNF, using a linear mixed effects model (all mRNA analyses followed the common procedure described in §4.3.5 unless otherwise specified), we found a main effect of Delay ($F(4, 172) = 2.733, p = .031$) and Region ($F(4, 172) = 167.069, p < .001$). The main effect of Region suggests that average BDNF mRNA levels were different between the brain regions. We also observed a two-way interaction of Region and Day ($F(12, 172) = 2.517, p = .004$) suggesting that average BDNF mRNA levels within each day of training differed between the regions. There were no other significant main effects or interactions, including interactions with Delay suggesting that mRNA levels did not increase significantly in response to multi-trial training.

In post-hoc comparisons, using the Bonferroni correction for multiple comparison, we found a general pattern that at most delays, across the days, BDNF

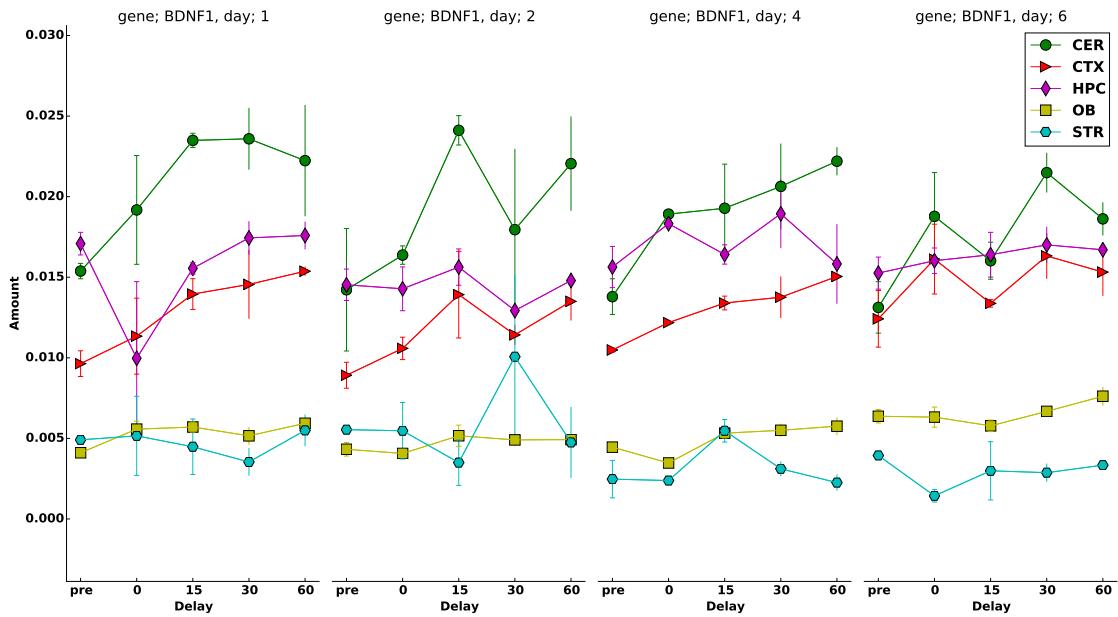


Figure 4.4: “Amount” is an arbitrary unit from BDNF mRNA transcript number normalized to the β -Actin mRNA number for the same delay and brain region. The lines represent different brain regions. BDNF mRNA transcript levels do not change in response to training on any of the days (all post-hoc comparisons of times to “pre” within Day and Regions were not significant, $p > .05$ for all comparisons). However, BDNF mRNA transcript levels were, in general, lower in the OB and STR relative to other regions (Table A.1).

mRNA levels in the OB and the STR were significantly lower than levels in the CTX ($p < .05$ for most comparisons), CER ($p < .05$ for most comparisons), and HPC ($p < .05$ for most comparisons). See Table A.1 for a complete report of the pairwise comparisons. OB and STR levels of BDNF mRNA did not differ in general ($p > .05$ for most comparisons). BDNF mRNA levels did not differ systematically between HPC, CER, and CTX ($p > .05$ for all but one comparison).

In general, BDNF mRNA transcript levels did not increase in response to training on any of the days, however, we do find that BDNF mRNA exists at

different levels between brain regions, with OB and STR having the least BDNF mRNA (Figure 4.4)

4.4.3 Intracellular signaling proteins, Erk 1 and Erk 2, and transcription factor, CREB, exist at different levels across brain regions

In general, we found that mRNA levels for three regulatory factors differ between regions, although there is no effect of learning on levels.

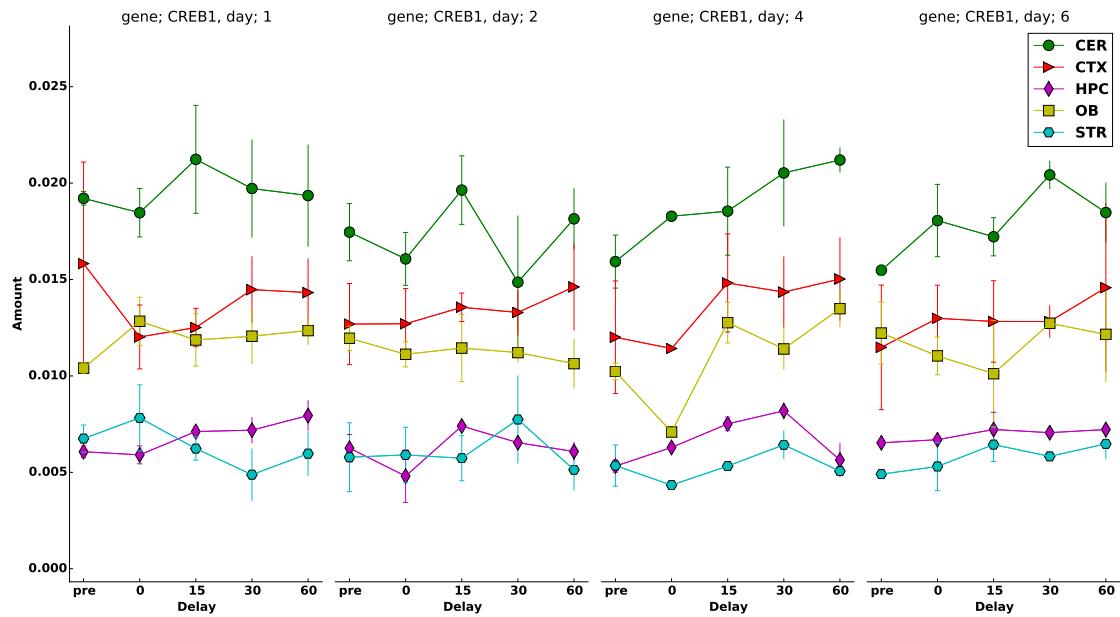


Figure 4.5: “Amount” is an arbitrary unit from CREB mRNA transcript number normalized to the β -Actin mRNA number for the same delay and brain region. The lines represent different brain regions. CREB mRNA transcript levels do not change in response to training on any of the days ($p > .05$ for most comparisons).

For CREB (Figure 4.5, our linear mixed effects model only showed a main

effect of Region ($F(4, 138.642) = 160.301, p < .001$) which suggests that on average CREB mRNA levels were different between the brain regions. There were no other significant main effects or interactions, including the main effect of Delay suggesting that mRNA levels did not increase significantly in response to multi-trial training.

In post-hoc comparisons, using the Bonferroni correction for multiple comparison, we found that CREB mRNA levels differed between brain regions during various delays across the days (Table A.2). Post-hoc tests also confirmed the finding that CREB mRNA levels did not change significantly after training, at any of the delays ($p > .05$ for most comparisons).

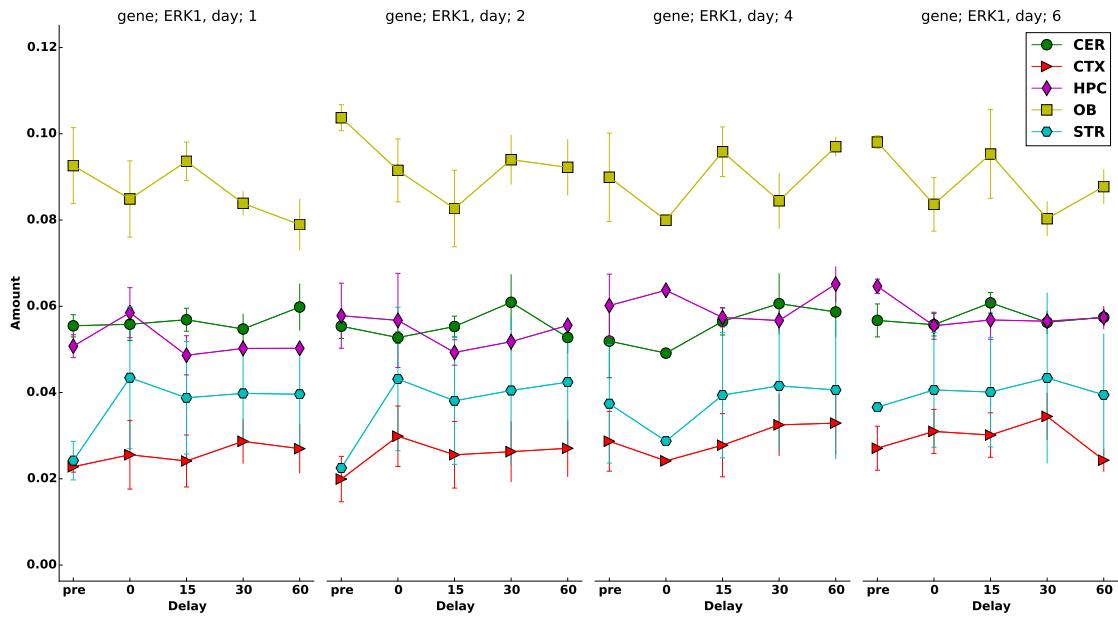


Figure 4.6: “Amount” is an arbitrary unit from Erk1 mRNA transcript number normalized to the β -Actin mRNA number for the same delay and brain region. Erk1 mRNA transcript levels do not change in response to training on any of the days ($p > .05$ for most comparisons).

For Erk 1 (Figure 4.6, our linear mixed effects model only showed a main

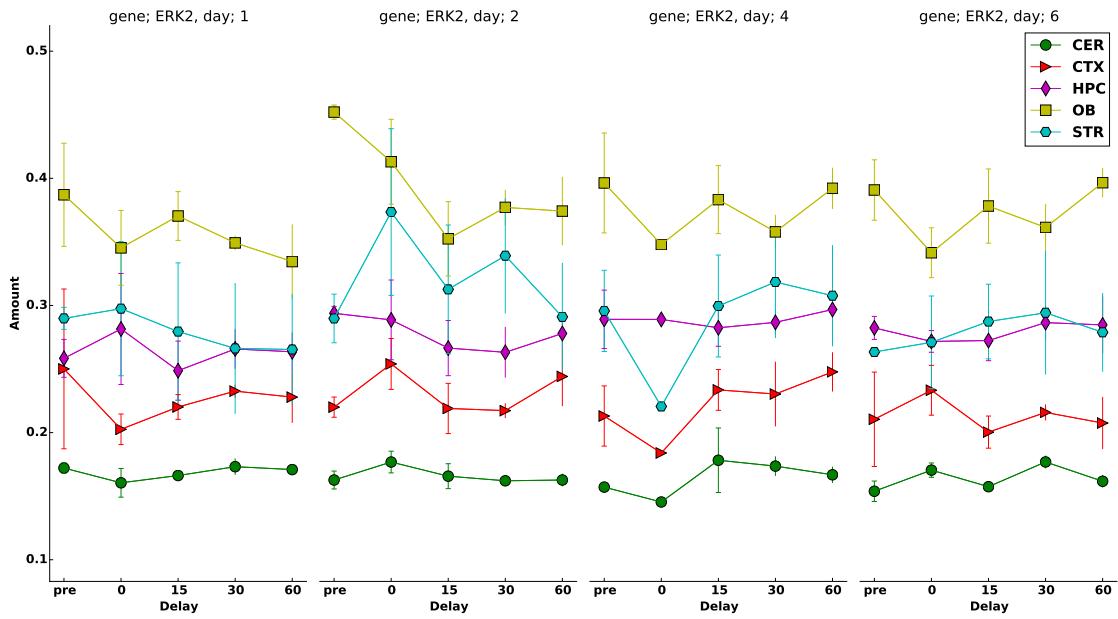


Figure 4.7: “Amount” is an arbitrary unit from Erk2 mRNA transcript number normalized to the β -Actin mRNA number for the same delay and brain region. The lines represent different brain regions. Erk2 mRNA transcript levels do not change in response to training on any of the days ($p > .05$ for most comparisons).

effect of Region ($F(4, 139.228) = 115.764, p < .001$) which suggests that on average Erk1 mRNA levels were different between the brain regions. There were no other significant main effects or interactions, including the main effect of Delay suggesting that mRNA levels did not increase significantly in response to multi-trial training.

In post-hoc comparisons, using the Bonferroni correction for multiple comparison, we found that Erk1 mRNA levels differed between brain regions during various delays across the days (Table A.3). In general, the levels are significantly higher in the OB ($p < .001$ for most comparisons). Post-hoc tests also confirmed the finding that Erk1 mRNA levels did not change significantly after training, at any of the delays ($p > .05$ for all comparisons).

For Erk 2 (Figure 4.7, our linear mixed effects model only showed a main effect of Region ($F(4, 173.000) = 191.291, p <.001$) which suggests that on average Erk2 mRNA levels differed between brain regions. There were no other significant main effects or interactions, including the main effect of Delay suggesting that mRNA levels did not increase significantly in response to multi-trial training.

As before, post-hoc comparisons using the Bonferroni correction showed that Erk2 mRNA levels differed between brain regions during various delays across the days (Table A.4). In general, the levels are significantly higher in the OB ($p <.05$ for most comparisons) for Erk1, however, notice that for Erk2 mRNA levels are much higher than Erk1. This confirms typical findings from other molecular neuroscience studies (unpublished data). Finally, post-hoc tests confirmed the finding that Erk2 mRNA levels did not change significantly after training, at any of the delays ($p >.05$ for most comparisons).

In summary, the results show that Erk 1, Erk 2, and CREB mRNA levels did not change significantly after training, however, they exist at different levels across brain regions.

4.4.4 Immediate Early Genes (IEGs) transcribed in response to learning

In general, the immediate early genes (IEGs), Arc, Egr1, and Fos, are newly transcribed following learning on each day. Timecourses differences by brain region and gene are outlined below.

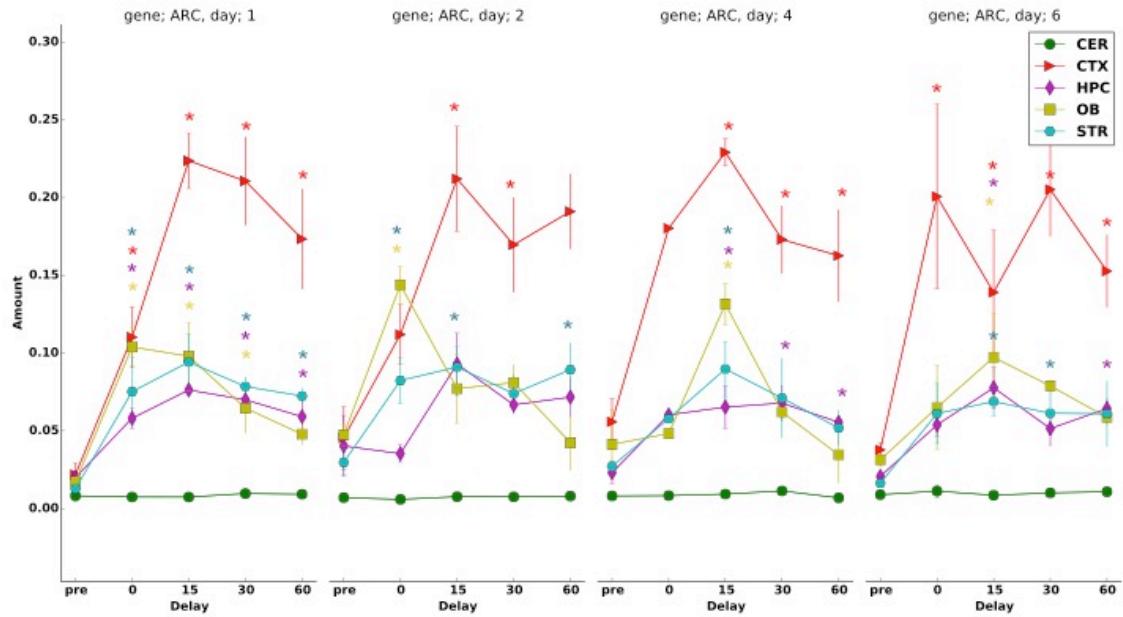


Figure 4.8: “Amount” is an arbitrary unit from Arc mRNA transcript number normalized to the β -Actin mRNA number for the same delay and brain region. The lines represent different brain regions. Arc mRNA transcript levels increase in response to training. Stars denote significant differences ($p <.05$) from the “pre” level of the same region on the same day.

For Arc, our linear mixed effects model yielded main effects of Delay ($F(4, 35.734) = 40.356, p <.001$) and Region ($F(4, 138.180) = 363.846, p <.001$). The main effect of Region suggests that average Arc mRNA levels differed between the brain regions. In addition, the main effect of Delay suggests that mRNA levels differed after learning. We observed a two-way interaction of Region and Delay ($F(16, 138.479) = 5.595, p <.001$) suggesting that average Arc timecourse differed between the regions. There were no other significant main effects or interactions.

In post-hoc comparisons, using the Bonferroni correction for multiple comparison, we found that for all brain regions, Arc mRNA levels significantly in-

creased after multi-trial learning at most delays and most days ($p < .05$ for most comparisons). Specific comparisons shown in Figure 4.8 and all comparisons shown on Table A.5. Together, we found that in general Arc mRNA levels increased robustly after multi-trial learning on each day and that the timecourse for Arc did not differ between days for most brain regions. For the HPC, however, the most robust learning-responsive activity seems to be on Day 1 and Day 4 of training.

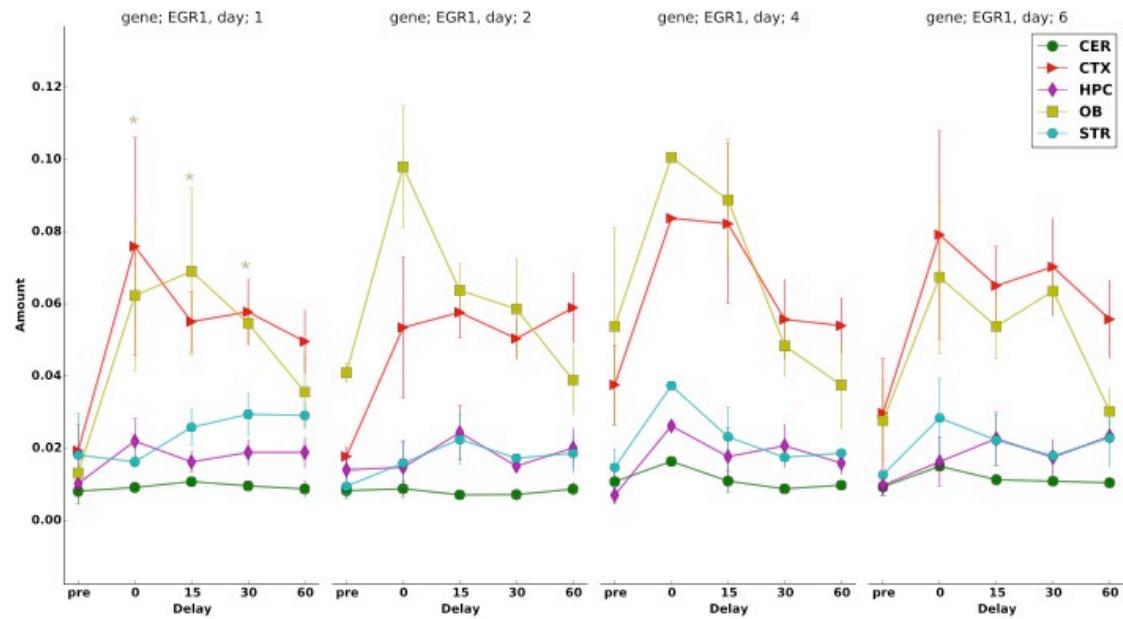


Figure 4.9: “Amount” is an arbitrary unit from Egr1 mRNA transcript number normalized to the β -Actin mRNA number for the same delay and brain region. The lines represent different brain regions. Egr1 mRNA transcript levels in the OB increase in response to training on Day 1. Stars denote significant differences ($p < .05$) from the “pre” level of the same region on the same day.

For the gene, Egr1, our linear mixed effects model yielded main effects of Delay ($F(4, 36.859) = 7.216, p < .001$) and Region ($F(4, 138.040) = 152.939, p < .001$). The main effect of Region suggests that average Egr1 mRNA levels differed

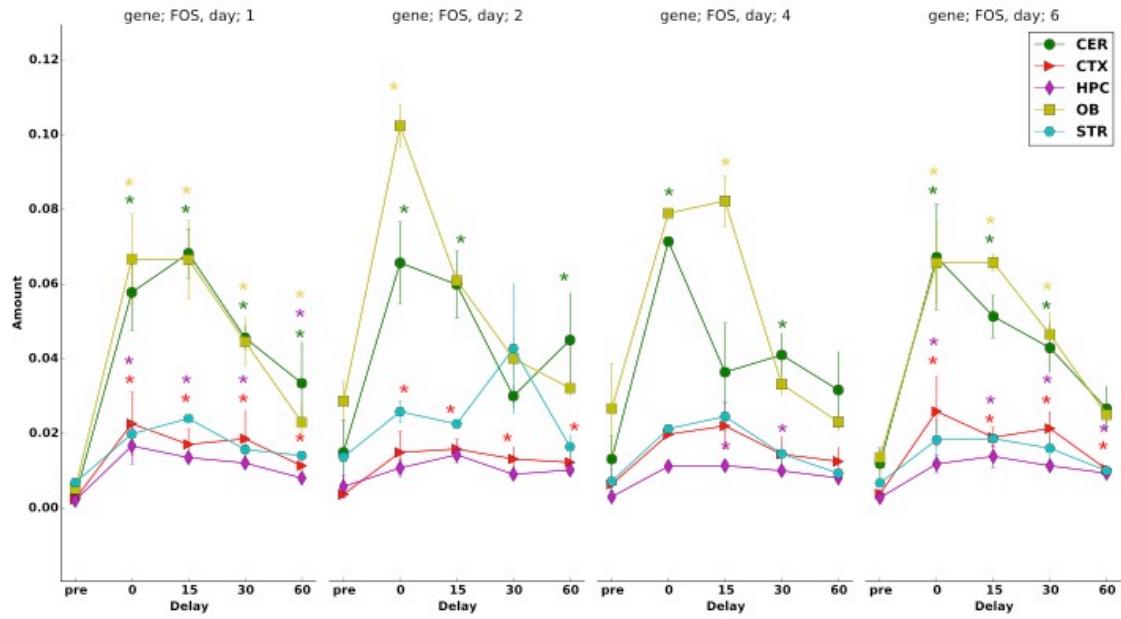


Figure 4.10: “Amount” is an arbitrary unit from Fos mRNA transcript number normalized to the β -Actin mRNA number for the same delay and brain region. The lines represent different brain regions. Fos mRNA transcript levels increase in response to training. Stars denote significant differences ($p <.05$) from the “pre” level of the same region on the same day.

between the brain regions. In addition, the main effect of Delay suggests that mRNA levels differed after learning. We observed a two-way interaction of Region and Delay ($F(16, 138.106) = 2.052, p = .014$) suggesting that average Egr1 timecourse differed between the regions. There were no other significant main effects or interactions.

In post-hoc comparisons, however, we found that Egr1 mRNA levels increased after learning only on Day 1 in the OB ($p <.05$ for all delays compared to “pre”). Specific comparisons shown in Figure 4.9 and shown on Table A.6. While increases in the OB were only significant on Day 1, Figure 4.9 shows patterns of increases, at least, in the OB and CTX for all four days measured.

For Fos, our linear mixed effects model again showed main effects of Delay ($F(4, 32.133) = 38.221, p <.001$) and Region ($F(4, 131.066) = 237.798, p <.001$). The main effect of Region suggests that average Fos mRNA levels differed between the brain regions. In addition, the main effect of Delay suggests that mRNA levels were influenced by learning. We observed a two-way interaction of Region and Delay ($F(16, 130.931) = 3.337, p <.001$) suggesting that average Fos time-course differed between the regions. We also observed a two-way interaction of Region and Day ($F(12, 131.024) = 3.138, p = .001$) suggesting that, on average, Fos mRNA levels differed between the regions as a function of the number of training days. There were no other significant main effects or interactions.

In post-hoc comparisons, using the Bonferroni correction for multiple comparison, we found that for all brain regions except the striatum, Fos mRNA levels significantly increased after the first day of multi-trial learning ($p <.05$ for all comparisons). For the OB, CER, and CTX, learning-responsive Fos mRNA transcript increases persisted on Day 2, 4, and 6. However, hippocampal Fos mRNA levels did not significantly change in response to learning on Day 2, and learning-responsiveness returns on Day 4 and 6. Specific comparisons shown in Figure 4.10 and shown on Table A.7. Together, we found that in general Fos mRNA levels increased after multi-trial learning, but this change depends on the brain region.

4.4.5 Regional differences in IEG activity

We observed regional differences in activity for the IEGs (Figure 4.11 shows an example of these patterns for Day 1) both in terms of expression and timecourse.

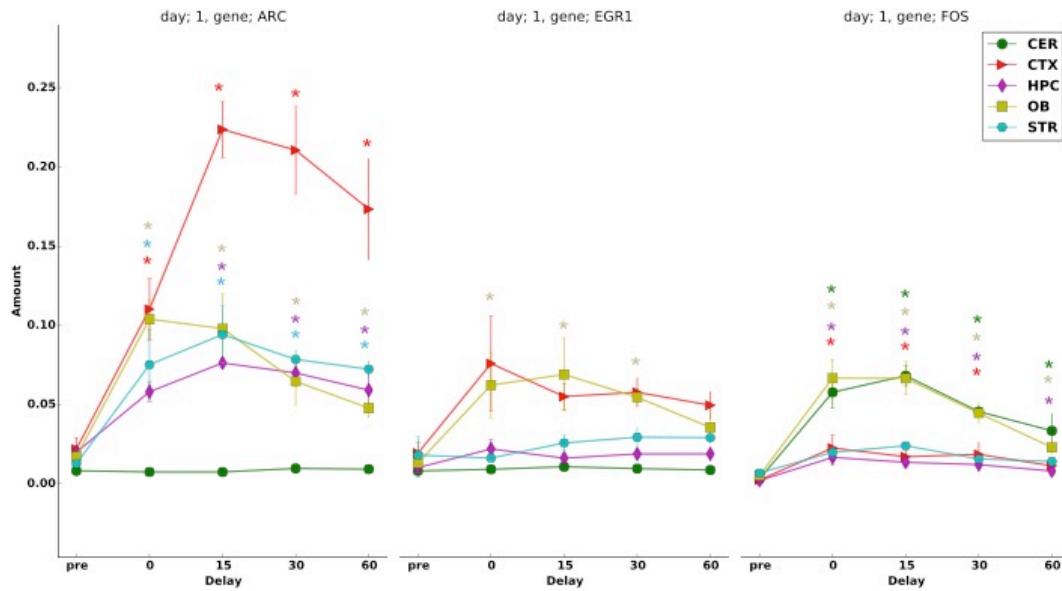


Figure 4.11: “Amount” is an arbitrary unit from the gene mRNA transcript number normalized to the β -Actin mRNA number for the same delay and brain region. Graph shows the timecourses for Arc, Egr1, and Fos on Day 1 of multi-trial training. An example of regional differences in IEG activity, both in terms of expression and timecourse. Stars represent significant increases above “pre” mRNA levels within a given brain region for that day ($p <.05$ these comparisons).

For expression differences in the OB, all IEGs showed significant mRNA transcript increase following learning (analyses described in the previous sections, Tables A.5, A.7, A.6). Pairwise comparisons of IEG mRNA levels in the OB at all delays on Day 1 were not significant ($p >.05$ for all comparisons, see Table A.8) suggesting that OB-specific IEG transcription responded similarly in degree to multi-trial training. In contrast, in the cerebellum, Fos mRNA levels were significantly higher at post-learning delays than Arc and Egr1 (textit{p} <.05 for most comparisons between Fos and the other IEGs, Table A.8).

4.5 Conclusion

In the current study, we trained animals on an associative odor-reward task over 6 days. We found that animals were able to acquire this association and reached asymptotic performance by Day 3 of training. Using high-throughput RT-PCR, we measured mRNA levels before and after training on each day. Below, I discuss the results for BDNF mRNA levels first and then address some more general findings from the study.

In previous chapters, we found that blockade of BDNF receptor activity in the OB during learning had negative effects on LTM and odor habituation. Thus, it makes sense to expect some learning-induced changes in BDNF mRNA in the OB following a similar associative training paradigm. However, we did not observe significant differences in mRNA BDNF levels after Day 1 of training. In fact, while we did observe regional differences in existing transcript levels (previous literature supports the idea that BDNF mRNA transcript levels exists that different levels between structures. For example, [122] found that total BDNF mRNA levels were lower in the OB than in the HPC, similarly to our study), we did not observe learning-induced changes in BDNF mRNA transcript levels in any of the brain regions we tested. Thus it is possible that the behavior we observed in Chapter 2 and Chapter 3 is sufficiently mediated by existing BDNF protein in the OB. Even more likely, however, is the idea that bulbar BDNF is produced largely by extrabulbar neurons and transported into the OB for release via axon transport. Under these circumstances, BDNF mRNA would not be transcribed within the OB, and therefore, there would be minimal mRNA within the OB for our analysis to detect. This idea is supported by some evidence suggesting that GABAergic interneurons, which make up the major-

ity of the OB, do not produce BDNF [58] although they are regulated by BDNF [129].

There are also some limitations of the current method that may explain the results. For example, it is possible that our method was simply not sensitive enough to account for the changes in the OB. The mouse *bdnf* gene spans approximately 50 kb of genomic DNA (for review, see [3]). Until recently, the gene was thought to consist of five exons, with only exon V encoding the entire BDNF protein and exon I-IV being noncoding segments. Each noncoding exon has an independent promoter and can be spliced directly to exon V [181]. However, more recent work finds that the different combinations of nine promoters and multiple exons, not just exon V, can produce a total of 24 different transcripts. Strangely, all these transcripts encode the same mature BDNF protein, a unique characteristic of BDNF that is suggestive of many levels of transcriptional regulation for *bdnf* [25] and a multiplicity of functions including tissue specificity for the protein [46]. Thus, a more sensitive mRNA analysis method that can look at differences in timecourse for the multitude of *bdnf* splice variants may be better suited to detect these more fine-grade changes.

It is also worth noting that studies indeed suggest that there are regional differences in the existence of BDNF and its many splice variants. Following a fear-potentiated startle paradigm, where the strength of a startle response to an acoustic stimulus is elevated in the presence of light that has been previously paired with a foot shock, BDNF mRNA levels were high in the basal lateral amygdala two hours following fear conditioning and returned to baseline levels by four hours after training. No changes in BDNF mRNA levels were observed in the HPC, medial nucleus of the amygdala or ventral posteromedial

nucleus of the thalamus [161] suggesting that endogenous BDNF activity in response to learning events is region-specific. Furthermore, [161] found a selective increase in BDNF transcripts containing exons I and III in the amygdala two hours following fear conditioning. In contrast, mRNA levels of BDNF exons II and IV remained unchanged. This suggests that there is some specificity in the mobilization of the different transcripts, which may have led to non-significant differences in our current study, but remains to be investigated.

Even beyond larger regional differences, BDNF mRNA transcript levels have been shown to differ between the subregions within a structure. Amongst the olfactory structures, for example, exon V is lower in mitral, granule, and periglomerular cell layers compared to the OSN layer, exon I-IV expression is low with exon I being the least dominant in this region. Expression of exon V is highest in the piriform cortex [122]. One major limitation of the current study is exactly that we used fairly gross anatomical dissections of whole regions. We know from many studies that subregion/cell-type specific changes can differ greatly in level, but also in time course. For example, in the nucleus accumbens, following a single cocaine injection, BDNF mRNA levels peak 1 hour after injection, but in the shell only, and not the core [70]. Another strong example is unpublished work from Luo and colleagues which show, using immunohistochemistry, that subregions within a single structure can differ greatly in EGR1 expression following olfactory conditioning. When the subregions are grouped together, the differences are no longer significant.

Thus, the absence of learning-induced changes in BDNF mRNA levels within the structures can be explained by the limitations of our current methodology to detect both inter-regional and intra-regional differences in BDNF alter-

native splicing and, possibly, time course [70]. Future studies need to use techniques which are more sensitive to alternative splicing to account for regional localization of the BDNF mRNA isoforms. This could paint a more complete picture of learning-induced molecular activity, as well as, contribute substantially to our understanding of the role of the detailed transcriptional control that allows the same molecular mechanism to underlie such diverse behaviors.

Other findings from the study were that intracellular signaling genes, Erk 1 and Erk2, and the transcription factor, CREB, exist at different levels across the brain, but do not significantly change in response to learning. These mechanisms participate in multiple pathways [118], including regulatory mechanisms, and it makes sense that their transcription was not greatly perturbed by learning. However, multiple studies of these pathways, including those reviewed in Chapter 1, have shown that these mechanisms are important and needed for LTM [171]. In order to better understand their roles, future studies will need to measure the activity levels of their proteins, using proteomic techniques or simple Western blotting, to more effectively understand the timecourse of their participation.

We also found that immediate early genes (IEGs), which have been used extensively as markers of learning-responsive brain regions, are indeed newly transcribed after learning. In general, we saw increases in Arc, Egr1, and Fos mRNA levels after multi-trial training on all the days. However, there were regional differences in their expression and timecourse. One interesting finding was that hippocampal levels of both Arc and Fos show a robust and sustained increase after the first day of learning, but not after the second day. This finding is in keeping with the idea of the HPC as a novelty detector [105]. Hippocampal

Fos and Arc mRNA levels do respond to training again on Day 4, however, this could be the response of a new neuronal population.

In summary, the strength of the current study is that it generated a “spatiotemporal map” of plasticity-related mechanisms following multi-trial learning. Future studies can use this “map” to more specifically probe the role of these mechanisms, as well as the importance of their timing. For example, we can ask questions about the functional role of Fos activity in the OB at a given time after learning, including behavioral and structural effects. In the present study, we observed increased in Fos mRNA in the OB immediately and 15 minutes after multi-trial training on all the days. Is this brief time frame crucial for LTM consolidation? We can test this by artificially elevating Fos levels in the OB following learning beyond 15-minutes and then using behavioral testing to assess the effect of this manipulation on LTM. More impactful, however, is the fact the map captures the timecourses of multiple brain regions for the same learning event. This allows us to test hypotheses about how brain regions may coordinate with each other for higher-order computations.

CHAPTER 5

GENERAL CONCLUSION

The central aim of this dissertation is to contribute to a more comprehensive understanding of the timecourse of plasticity-related mechanisms, like the BDNF-TrkB pathway, in temporally distributed learning. This work is motivated by now-canonical findings, using one-trial learning paradigms, which show that STM and LTM pathways may be independent from the outset, with LTM being *de novo* protein synthesis-dependent and STM being protein synthesis-independent. A broader implication of this result challenges our intuition that STM becomes LTM. Instead, it implies that the “decision” of whether a learning event is consolidated for LTM or not, actually occurs very shortly after the event itself. Thus, examining the molecular mechanisms involved in the early, post-learning period is a means by which we can understand how the brain makes predictions about the long-term utility of acquired information.

In the second chapter, we observed that for a multi-trial, associative learning task, BDNF receptor activity in the OB was needed, around the time of acquisition, for LTM but not STM. The LTM deficit in animals that received the receptor blockade was associated with low certainty for the rewarded odor, suggesting that indeed some memory-related mechanism was disrupted. From the one-trial learning literature, we saw that protein synthesis inhibition not just in the HPC, but also the amygdala and entorhinal cortex disrupted LTM, but not STM. The fact that this STM/LTM dissociation is observed following protein synthesis inhibition in various regions, including a primary sensory region (from Chapter 2), eludes to the idea that the “decision” to consolidate arises primarily from properties of the synapses involved, rather than a higher-order computation.

However, work still remains to be done to examine this hypothesis directly. Additionally, the idea does not exclude the importance of understanding interactions between regions. For example, from the Selectivity Index in Chapter 2, we see that although drug animals showed low certainty during their first trial of LTM testing, their performance increases to normal levels by Trial 5 suggesting that some extra-bulbar retention of the odor-reward association was preserved.

A general theme of this dissertation is the interest in the coordination of multiple mechanisms across multiple brain regions. This motivated the design of the experiment in Chapter 4, but the idea of interaction is also a framework for examining the results of Chapter 3. In Chapter 3, we found first that a baseline level of BDNF is needed in the developing brain for normal representational learning in adulthood and that, importantly, acute, OB-specific blockade of BDNF receptors did not interfere with the formation of specific odor representations. We additionally observed a trend (albeit not statistically significant) that BDNF receptor blockade prevented odor habituation, a behavioral observation that is also seen when NDMAR activity is inhibited.

This is an interesting parallel, because the limited work looking at the interaction between TrkB and NMDA receptors supports the idea that they together coordinate LTM consolidation. First, TrkB and NMDARs can be found within the same post-synaptic densities [194, 175]. Second, there is evidence for their interaction in LTP. [114] found that *in vitro*, bath application of BDNF with cortical or hippocampal postsynaptic densities increased tyrosine phosphorylation of the NR2B subunits in a dose-dependent manner. Work from Yamada and colleagues [195, 195] suggests that TrkB and NMDARs interact through tyrosine kinase Fyn and this interaction is important for behavioral memory

performance. [141] found that radial arm maze training induced phosphorylation of TrkB, Fyn, and NR2B in the hippocampus. It did not, however, induce changes in NMDA's NR2A subunit, a pattern similar to the one found *in vitro* by [114]. The authors also found that TrkB, NR2B, and Fyn were more likely to co-immunoprecipitated in well-trained rats than in controls, suggesting that their co-activity was important for learning. Infusion of PP2, a tyrosine kinase inhibitor, led to diminished phosphorylation of Fyn and NR2B, but not TrkB. This final finding suggests that the TrkB receptor is directly responsive to neuronal activity and may be the initiating step of the TrkB/NMDA-mediated synaptic changes. However, further studies are needed to examine the exact nature of the changes.

One hypothesis merges the role of NDMA receptors in LTP with the BDNF/TrkB pathway's role as a synaptic tag (discussed in §3.5) as a mechanism of synaptic specificity. That is, that NMDAR activity initiates E-LTP broadly across all activated synapses, but TrkB activity tags specific synapses for the protein-synthesis mediated transition to L-LTP. In support of this idea, [120] find a transient increase in pTrkB levels shortly following LTP induction and this increase is, crucially, protein-synthesis independent. Blockade of this protein-synthesis independent peak thirty minutes after E-LTP induction prevented the induction of L-LTP [120] suggesting that this early TrkB activity is needed for the transition to longer lasting forms of plasticity. Additional studies have found that BDNF is needed for LTP induction [103, 119, 14, 120, 93], specifically that BDNF alone in the presence of protein synthesis inhibitors is sufficient to induce L-LTP from E-LTP [119]. LTP induction also requires NMDA receptor activation. However, the key result in support of our idea is that in order for NMDAR-dependent LTP to last beyond 4 hours, it requires protein synthesis

and is permitted by BDNF [92].

Finally, in Chapter 4, we generated a “spatiotemporal map” of PRP following multi-trial learning which can be useful for testing specific hypotheses. The temporal patterns from our “map”, together with the finding in Chapter 2 that a STM/LTM dissociation is observed for multi-trial learning paradigms, suggest that the timecourse of some of the early, post-learning mechanisms are quite similar for both one-trial and multi-trial learning. For example, in Fig.1.2 we see that many PRPs are highly responsive within 30 minutes of the learning trial. In Chapter 4, we saw that IEG activity in several brain regions showed high learning-responsive levels by 15- and 30-minutes after training that decreased by 60 minutes. The observation that the timecourse for these mechanisms are so similar between one-trial and multi-trial learning paradigms suggests to me that these mechanisms may not necessarily be responding to individual parameters of learning, but rather use the parameters to determine the long-term utility of a learning *event*. That is, the unit of long-term consolidation is the “event”. Indeed, a body of work examining event segmentation in humans has shown that memory for information is a function of the occurrence of perceptual event boundaries [106, 179]. Thus, a complete understanding of LTM consolidation will require the integration of the presently separate literatures in the molecular mechanisms of memory and in event segmentation.

APPENDIX A

TABLES OF FULL POST-HOC COMPARISONS FROM CHAPTER 4

NOTE on repetition in the standard errors reported in these tables: This occurs because the pairwise comparisons were performed using estimated marginal means (EMM) rather than group means. Beginning with the full mixed effects analysis, the mixed model function uses my data to fit a new model, and uses the fitted model for comparisons. This means that it makes estimates of the group means as well as standard error. So although the model generates a different mean for each group, the model assumes that variation about the mean is the same for all the groups (i.e. the homogeneity of variance assumption). The EMM are generated from the full model estimates and therefore the standard error reflects the estimates of the model. For each group mean, the single estimated standard error is multiplied by a constant (which depends on the number of factors in the full model). Thus, the means will all have the same standard error only if all the groups have an equal number of cases. Therefore, repetition of standard errors in the table below are normal for the method of analysis and indicate comparisons for which the groups being compared have the same number of cases.

Table A.1: The full table of post-hoc pairwise comparisons for BDNF mRNA, discussed in §4.4.2. Bonferroni correction for multiple comparisons was used. P-values (“Sig.”) are reported with the standard error (“Std. Error”). Mean differences for which p-values less than .05 are starred for emphasis.

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
pre	D1	OB	HPC	-1.425*	.393	.004
			CTX	-.850	.393	.319
			STR	-.179	.481	1.000
			CER	-1.320*	.393	.010
	HPC	OB	OB	1.425*	.393	.004
			CTX	.575	.393	1.000
			STR	1.246	.481	.104
			CER	.105	.393	1.000
	CTX	OB	OB	.850	.393	.319
			HPC	-.575	.393	1.000
			STR	.671	.481	1.000
			CER	-.470	.393	1.000
	STR	OB	OB	.179	.481	1.000
			HPC	-1.246	.481	.104
			CTX	-.671	.481	1.000
			CER	-1.141	.481	.187
	CER	OB		1.320*	.393	.010

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D2	OB	HPC	HPC	-.105	.393	1.000
			CTX	.470	.393	1.000
			STR	1.141	.481	.187
		OB	HPC	-1.215*	.393	.023
	HPC	CTX	CTX	-.725	.393	.668
		STR	STR	-.252	.393	1.000
		CER	CER	-1.158*	.393	.036
		OB	OB	1.215*	.393	.023
	CTX	CTX	OB	.490	.393	1.000
		STR	STR	.962	.393	.153
		CER	CER	.056	.393	1.000
		OB	OB	.725	.393	.668
D4	STR	HPC	HPC	-.490	.393	1.000
			STR	.472	.393	1.000
			CER	-.434	.393	1.000
		OB	OB	.252	.393	1.000
	CER	STR	HPC	-.962	.393	.153
		STR	CTX	-.472	.393	1.000
		STR	CER	-.906	.393	.223
		OB	OB	1.158*	.393	.036
	OB	CER	HPC	-.056	.393	1.000
		CER	CTX	.434	.393	1.000
		CER	STR	.906	.393	.223
		OB	HPC	-1.250*	.321	.001
		OB	CTX	-.857	.321	.083

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	HPC	STR	.712	.358	.484
			CER	-1.128*	.358	.020
		CTX	OB	1.250*	.321	.001
			STR	.393	.321	1.000
			CER	1.962*	.358	.000
			OB	.122	.358	1.000
	CTX	STR	OB	.857	.321	.083
			HPC	-.393	.321	1.000
		CER	STR	1.569*	.358	.000
			OB	-.271	.358	1.000
			STR	-.712	.358	.484
	CER	STR	OB	-1.962*	.358	.000
			HPC	-1.569*	.358	.000
		CTX	OB	-.122	.358	1.000
			STR	.271	.358	1.000
			CER	-1.840*	.393	.000
	D6	OB	OB	1.128*	.358	.020
			HPC	-.122	.358	1.000
			STR	.271	.358	1.000
			CER	1.840*	.393	.000
		STR	OB	-.873	.393	.275
		CTX	OB	-.659	.393	.950
		STR	OB	.478	.481	1.000
		CER	OB	-.717	.393	.695
		HPC	OB	.873	.393	.275
		CTX	OB	.214	.393	1.000
		STR	OB	1.351	.481	.055

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
0min	D1	OB	CER	.156	.393	1.000
			CTX	.659	.393	.950
			HPC	-.214	.393	1.000
			STR	1.137	.481	.191
		STR	CER	-.058	.393	1.000
			OB	-.478	.481	1.000
			HPC	-1.351	.481	.055
			CTX	-1.137	.481	.191
		CER	CER	-1.196	.481	.139
			OB	.717	.393	.695
			HPC	-.156	.393	1.000
			CTX	.058	.393	1.000
		HPC	STR	1.196	.481	.139
			OB	.095	.321	1.000
			CTX	-.672	.321	.375
			STR	.277	.321	1.000
		STR	CER	-1.213*	.321	.002
			OB	-.095	.321	1.000
			CTX	-.767	.321	.178
			STR	.183	.321	1.000
		CTX	CER	-1.308*	.321	.001
			OB	.672	.321	.375
			HPC	.767	.321	.178
			STR	.950*	.321	.035
		CER	CER	-.541	.321	.933

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	STR	OB	-.277	.321	1.000
			HPC	-.183	.321	1.000
			CTX	-.950*	.321	.035
			CER	-1.491*	.321	.000
	Day 2	CER	OB	1.213*	.321	.002
			HPC	1.308*	.321	.001
			CTX	.541	.321	.933
			STR	1.491*	.321	.000
	Day 3	OB	HPC	-1.252*	.321	.001
			CTX	-.957*	.321	.033
			STR	-.205	.321	1.000
			CER	-1.396*	.321	.000
	Day 4	HPC	OB	1.252*	.321	.001
			CTX	.295	.321	1.000
			STR	1.047*	.321	.013
			CER	-.144	.321	1.000
	Day 5	CTX	OB	.957*	.321	.033
			HPC	-.295	.321	1.000
			STR	.751	.321	.202
			CER	-.439	.321	1.000
	Day 6	STR	OB	.205	.321	1.000
			HPC	-1.047*	.321	.013
			CTX	-.751	.321	.202
			CER	-1.191*	.321	.003
	Day 7	CER	OB	1.396*	.321	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.	
D4	OB	HPC	HPC	.144	.321	1.000	
			CTX	.439	.321	1.000	
			STR	1.191*	.321	.003	
		CER	HPC	-1.663*	.555	.032	
	HPC		CTX	-1.255	.555	.251	
			STR	.377	.555	1.000	
			CER	-1.695*	.555	.026	
	CTX	OB	OB	1.663*	.555	.032	
		STR	CTX	.408	.555	1.000	
			STR	2.040*	.555	.003	
			CER	-.032	.555	1.000	
	STR	OB	OB	1.255	.555	.251	
		CER	HPC	-.408	.555	1.000	
			STR	1.631*	.555	.038	
			CER	-.440	.555	1.000	
D6	OB	OB	OB	-.377	.555	1.000	
		HPC	HPC	-2.040*	.555	.003	
			CTX	-1.631*	.555	.038	
		CER	CER	-2.071*	.555	.003	
	HPC	OB	OB	1.695*	.555	.026	
		STR	HPC	.032	.555	1.000	
			CTX	.440	.555	1.000	
			STR	2.071*	.555	.003	
	OB	HPC	HPC	-.937*	.321	.039	
			CTX	-.929*	.321	.043	

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
15min	D1	HPC	STR	1.564*	.321	.000
			CER	-1.078*	.321	.009
		CTX	OB	.937*	.321	.039
			STR	.008	.321	1.000
		STR	STR	2.501*	.321	.000
			CER	-.141	.321	1.000
			OB	.929*	.321	.043
			HPC	-.008	.321	1.000
			STR	2.493*	.321	.000
	CER	STR	CER	-.150	.321	1.000
			OB	-1.564*	.321	.000
		CTX	HPC	-2.501*	.321	.000
			STR	-2.493*	.321	.000
		CER	CER	-2.642*	.321	.000
			OB	1.078*	.321	.009
			HPC	.141	.321	1.000
			CTX	.150	.321	1.000
			STR	2.642*	.321	.000
15min	D1	OB	HPC	-1.005*	.321	.020
			CTX	-.893	.321	.060
			STR	.487	.321	1.000
			CER	-1.418*	.321	.000
			HPC	1.005*	.321	.020
	D2	HPC	CTX	.112	.321	1.000
			STR	1.492*	.321	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	CTX	CER	-.413	.321	1.000
			OB	.893	.321	.060
			HPC	-.112	.321	1.000
		STR		1.380*	.321	.000
	Day 2	STR	CER	-.525	.321	1.000
			OB	-.487	.321	1.000
			HPC	-1.492*	.321	.000
		CTX		-1.380*	.321	.000
	Day 3	CER	STR	-1.905*	.321	.000
			OB	1.418*	.321	.000
			HPC	.413	.321	1.000
		CTX		.525	.321	1.000
		STR		1.905*	.321	.000
D2	Day 1	OB	HPC	-1.118*	.321	.006
			CTX	-.971*	.321	.028
			STR	.663	.321	.401
			CER	-1.555*	.321	.000
	Day 2	HPC	OB	1.118*	.321	.006
			CTX	.146	.321	1.000
			STR	1.781*	.321	.000
			CER	-.437	.321	1.000
	Day 3	CTX	OB	.971*	.321	.028
			HPC	-.146	.321	1.000
			STR	1.634*	.321	.000
			CER	-.584	.321	.703

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D4	OB	STR	OB	-.663	.321	.401
			HPC	-1.781*	.321	.000
			CTX	-1.634*	.321	.000
			CER	-2.218*	.321	.000
	CER	CER	OB	1.555*	.321	.000
			HPC	.437	.321	1.000
			CTX	.584	.321	.703
			STR	2.218*	.321	.000
	HPC	OB	HPC	-1.127*	.321	.006
			CTX	-.925*	.321	.044
			STR	-.013	.321	1.000
			CER	-1.257*	.300	.000
	CTX	OB	OB	1.127*	.321	.006
			CTX	.203	.321	1.000
			STR	1.115*	.321	.006
			CER	-.130	.300	1.000
	STR	OB	OB	.925*	.321	.044
			HPC	-.203	.321	1.000
			STR	.912*	.321	.050
			CER	-.332	.300	1.000
	CER	OB	OB	.013	.321	1.000
			HPC	-1.115*	.321	.006
			CTX	-.912*	.321	.050
			CER	-1.245*	.300	.001

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	HPC	HPC	.130	.300	1.000
			CTX	.332	.300	1.000
			STR	1.245*	.300	.001
		OB	HPC	-1.040*	.321	.014
	HPC	CTX	CTX	-.843	.321	.093
		STR	STR	1.025*	.321	.017
		CER	CER	-1.018*	.321	.018
		OB	OB	1.040*	.321	.014
	CTX	CTX	CTX	.197	.321	1.000
		STR	STR	2.065*	.321	.000
		CER	CER	.022	.321	1.000
		OB	OB	.843	.321	.093
30min	D1	HPC	HPC	-.197	.321	1.000
			STR	1.868*	.321	.000
			CER	-.175	.321	1.000
		OB	OB	-1.025*	.321	.017
	STR	HPC	HPC	-2.065*	.321	.000
		CTX	CTX	-1.868*	.321	.000
		CER	CER	-2.043*	.321	.000
		OB	OB	1.018*	.321	.018
	CER	HPC	HPC	-.022	.321	1.000
		CTX	CTX	.175	.321	1.000
		STR	STR	2.043*	.321	.000
		OB	OB	-1.226*	.321	.002

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	HPC	STR		.443	.321	1.000
		CER		-1.524*	.321	.000
		OB		1.226*	.321	.002
		CTX		.199	.321	1.000
		STR		1.668*	.321	.000
	CTX	CER		-.298	.321	1.000
		OB		1.027*	.321	.016
		HPC		-.199	.321	1.000
		STR		1.469*	.321	.000
		CER		-.497	.321	1.000
D2	STR	OB		-.443	.321	1.000
		HPC		-1.668*	.321	.000
		CTX		-1.469*	.321	.000
		CER		-1.967*	.321	.000
		OB		1.524*	.321	.000
	CER	HPC		.298	.321	1.000
		CTX		.497	.321	1.000
		STR		1.967*	.321	.000
		OB		-.976*	.321	.027
		CTX		-.849	.321	.089
D3	STR	STR		-.487	.321	1.000
		CER		-1.213*	.321	.002
		OB		.976*	.321	.027
	HPC	CTX		.127	.321	1.000
		STR		.489	.321	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	CTX	CER	-.237	.321	1.000
			OB	.849	.321	.089
			HPC	-.127	.321	1.000
		STR	STR	.362	.321	1.000
			CER	-.364	.321	1.000
	Day 2	STR	OB	.487	.321	1.000
			HPC	-.489	.321	1.000
			CTX	-.362	.321	1.000
		CER	STR	-.726	.321	.249
			OB	1.213*	.321	.002
D4	Day 4	OB	HPC	-1.228*	.321	.002
			CTX	-.913*	.321	.049
			STR	.588	.321	.682
		CER	STR	-1.309*	.321	.001
			OB	1.228*	.321	.002
	Day 7	HPC	CTX	.315	.321	1.000
			STR	1.816*	.321	.000
			CER	-.081	.321	1.000
		CTX	OB	.913*	.321	.049
			HPC	-.315	.321	1.000
		STR	STR	1.502*	.321	.000
			CER	-.396	.321	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	STR	OB	-.588	.321	.682
			HPC	-1.816*	.321	.000
			CTX	-1.502*	.321	.000
			CER	-1.897*	.321	.000
	CER	STR	OB	1.309*	.321	.001
			HPC	.081	.321	1.000
			CTX	.396	.321	1.000
			STR	1.897*	.321	.000
	HPC	OB	OB	-.931*	.321	.042
			CTX	-.884*	.300	.037
			STR	.886	.321	.064
			CER	-1.166*	.321	.004
	CTX	OB	OB	.931*	.321	.042
			CTX	.047	.300	1.000
			STR	1.817*	.321	.000
			CER	-.235	.321	1.000
	STR	OB	OB	.884*	.300	.037
			HPC	-.047	.300	1.000
			STR	1.769*	.300	.000
			CER	-.282	.300	1.000
	CER	OB	OB	-.886	.321	.064
			HPC	-1.817*	.321	.000
			CTX	-1.769*	.300	.000
			CER	-2.052*	.321	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
60min	D1	OB	HPC	.235	.321	1.000
			CTX	.282	.300	1.000
			STR	2.052*	.321	.000
			HPC	-1.086*	.321	.009
		CER	CTX	-.953*	.321	.034
			STR	.110	.321	1.000
	D2	HPC	CER	-1.298*	.321	.001
			OB	1.086*	.321	.009
			CTX	.133	.321	1.000
			STR	1.196*	.321	.003
		CTX	CER	-.212	.321	1.000
			OB	.953*	.321	.034
			HPC	-.133	.321	1.000
	D2	STR	STR	1.063*	.321	.011
			CER	-.345	.321	1.000
			OB	-.110	.321	1.000
			HPC	-1.196*	.321	.003
		CER	CTX	-1.063*	.321	.011
			CER	-1.407*	.321	.000
			OB	1.298*	.321	.001
	D2	HPC	HPC	.212	.321	1.000
			CTX	.345	.321	1.000
			STR	1.407*	.321	.000
		OB	HPC	-1.101*	.321	.007
		CTX	CTX	-1.003*	.321	.021

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	HPC	STR		.457	.321	1.000
		CER		-1.481*	.321	.000
	CTX	OB		1.101*	.321	.007
		CTX		.099	.321	1.000
		STR		1.559*	.321	.000
		CER		-.380	.321	1.000
	STR	OB		1.003*	.321	.021
		HPC		-.099	.321	1.000
		STR		1.460*	.321	.000
		CER		-.479	.321	1.000
		OB		-.457	.321	1.000
D4	OB	HPC		-1.559*	.321	.000
		CTX		-1.460*	.321	.000
		CER		-1.938*	.321	.000
		OB		1.481*	.321	.000
		HPC		.380	.321	1.000
	HPC	CTX		.479	.321	1.000
		STR		1.938*	.321	.000
		OB		-.995*	.321	.022
		CTX		-.970*	.321	.029
		STR		.986*	.321	.024
		CER		-1.357*	.321	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	Day 1	CER	CER	-.363	.321	1.000
			CTX	.970*	.321	.029
			HPC	-.025	.321	1.000
			STR	1.956*	.321	.000
	Day 2	STR	CER	-.388	.321	1.000
			OB	-.986*	.321	.024
			HPC	-1.981*	.321	.000
			CTX	-1.956*	.321	.000
	Day 3	CER	CER	-2.344*	.321	.000
			OB	1.357*	.321	.000
			HPC	.363	.321	1.000
			CTX	.388	.321	1.000
	Day 4	STR	STR	2.344*	.321	.000
			OB	-.791	.321	.146
			CTX	-.700	.358	.525
			STR	.825	.321	.109
	Day 5	CER	CER	-.897	.321	.057
			OB	.791	.321	.146
			CTX	.091	.358	1.000
			STR	1.616*	.321	.000
	Day 6	HPC	CER	-.106	.321	1.000
			OB	.700	.358	.525
			CTX	-.091	.358	1.000
			STR	1.525*	.358	.000
	Day 7	CER	CER	-.197	.358	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
	STR	OB		-.825	.321	.109
		HPC		-1.616*	.321	.000
		CTX		-1.525*	.358	.000
		CER		-1.722*	.321	.000
	CER	OB		.897	.321	.057
		HPC		.106	.321	1.000
		CTX		.197	.358	1.000
		STR		1.722*	.321	.000

Table A.2: The full table of post-hoc pairwise comparisons for CREB mRNA, discussed in §4.4.3. Bonferroni correction for multiple comparisons was used. P-values (“Sig.”) are reported with the standard error (“Std. Error”). Mean differences for which p-values less than .05 are starred for emphasis.

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
pre	D1	OB	HPC	.540	.259	.389
			CTX	-.361	.259	1.000
			STR	-.981*	.259	.002
		CER		-.614	.259	.191
	HPC	OB		-.540	.259	.389
		CTX		-.901*	.259	.007

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	STR	STR	-1.521*	.259	.000
			CER	-1.153*	.259	.000
		CTX	OB	.361	.259	1.000
			HPC	.901*	.259	.007
		STR	STR	-.620	.259	.180
			CER	-.252	.259	1.000
			OB	.981*	.259	.002
		CER	HPC	1.521*	.259	.000
			CTX	.620	.259	.180
			CER	.368	.259	1.000
			OB	.614	.259	.191
	Day 2	STR	HPC	1.153*	.259	.000
			CTX	.252	.259	1.000
			STR	-.368	.259	1.000
			OB	.653	.259	.128
			CTX	-.047	.259	1.000
		HPC	STR	.775*	.259	.033
			CER	-.377	.259	1.000
		CTX	OB	-.653	.259	.128
			CTX	-.700	.259	.077
			STR	.122	.259	1.000
			CER	-1.029*	.259	.001
			OB	.047	.259	1.000
		STR	HPC	.700	.259	.077
			STR	.822*	.259	.018

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D4	OB	CER	CER	-.329	.259	1.000
			STR	-.775*	.259	.033
			HPC	-.122	.259	1.000
			CTX	-.822*	.259	.018
	CER	OB	CER	-1.151*	.259	.000
			STR	.377	.259	1.000
			HPC	1.029*	.259	.001
			CTX	.329	.259	1.000
	HPC	OB	STR	1.151*	.259	.000
			CER	.655*	.211	.024
			CTX	-.107	.211	1.000
			STR	.683*	.211	.015
D4	CTX	CER	CER	-.409	.239	.894
			STR	-.655*	.211	.024
			CTX	-.761*	.211	.004
			STR	.028	.211	1.000
	STR	OB	CER	-1.064*	.239	.000
			STR	.107	.211	1.000
			CTX	.761*	.211	.004
			STR	.790*	.211	.003
	STR	CER	CER	-.303	.239	1.000
			OB	-.683*	.211	.015
			HPC	-.028	.211	1.000
			CTX	-.790*	.211	.003

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	CER	OB	.409	.239	.894
			HPC	1.064*	.239	.000
			CTX	.303	.239	1.000
			STR	1.092*	.239	.000
	HPC	OB	OB	.618	.259	.183
			CTX	.095	.259	1.000
			STR	.849	.324	.097
			CER	-.245	.259	1.000
	CTX	OB	OB	-.618	.259	.183
			CTX	-.523	.259	.452
			STR	.231	.324	1.000
			CER	-.863*	.259	.011
	STR	OB	OB	-.095	.259	1.000
			HPC	.523	.259	.452
			STR	.754	.324	.214
			CER	-.340	.259	1.000
	CER	OB	OB	-.849	.324	.097
			HPC	-.231	.324	1.000
			CTX	-.754	.324	.214
			CER	-1.094*	.324	.009
	0min	OB	OB	.245	.259	1.000
			HPC	.863*	.259	.011
			CTX	.340	.259	1.000
			STR	1.094*	.324	.009
	D1			.773*	.211	.004

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
HPC	Day 1	CTX	OB	.074	.211	1.000
			STR	.529	.211	.134
			CER	-.368	.211	.837
	Day 2	CTX	OB	-.773*	.211	.004
			STR	-.699*	.211	.012
		CER	OB	-.244	.211	1.000
			STR	-1.141*	.211	.000
	Day 3	CTX	OB	-.074	.211	1.000
			STR	.699*	.211	.012
		CER	OB	.455	.211	.331
			STR	-.443	.211	.381
	Day 4	STR	OB	-.529	.211	.134
			STR	.244	.211	1.000
		CER	OB	-.455	.211	.331
			STR	-.898*	.211	.000
	Day 5	CER	OB	.368	.211	.837
			STR	1.141*	.211	.000
		D2	OB	.443	.211	.381
			STR	.898*	.211	.000
	Day 6	HPC	OB	.938*	.211	.000
			STR	-.114	.211	1.000
			CER	.684*	.211	.015
			OB	-.364	.211	.875
	Day 7	HPC	OB	-.938*	.211	.000
			STR	-1.052*	.211	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	STR	OB	-.254	.211	1.000
			CER	-1.301*	.211	.000
		CTX	OB	.114	.211	1.000
			HPC	1.052*	.211	.000
			STR	.798*	.211	.002
	Day 2	CER	OB	-.249	.211	1.000
			STR	-.684*	.211	.015
		HPC	OB	.254	.211	1.000
			CTX	-.798*	.211	.002
			CER	-1.048*	.211	.000
D4	Day 4	CER	OB	.364	.211	.875
			HPC	1.301*	.211	.000
		STR	OB	.249	.211	1.000
			STR	1.048*	.211	.000
			OB	.120	.366	1.000
	Day 5	OB	CTX	-.477	.366	1.000
			STR	.492	.366	1.000
		CER	CTX	-.947	.366	.107
			STR	-.120	.366	1.000
			OB	-.596	.366	1.000
D7	Day 7	HPC	STR	.372	.366	1.000
			CER	-1.067*	.366	.042
			OB	.477	.366	1.000
		STR	CTX	.596	.366	1.000
			OB	.969	.366	.091

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	CER	CER	-.470	.366	1.000
			STR	-.492	.366	1.000
		HPC	HPC	-.372	.366	1.000
			CTX	-.969	.366	.091
			CER	-1.439*	.366	.001
	HPC	CER	OB	.947	.366	.107
			HPC	1.067*	.366	.042
		CTX	CTX	.470	.366	1.000
			STR	1.439*	.366	.001
			OB	.493	.211	.211
D6	CTX	HPC	CTX	-.151	.211	1.000
			STR	.780*	.211	.003
		CER	CER	-.490	.211	.218
			OB	-.493	.211	.211
			CTX	-.644*	.211	.028
	STR	HPC	STR	.287	.211	1.000
			CER	-.983*	.211	.000
		OB	OB	.151	.211	1.000
			HPC	.644*	.211	.028
			STR	.931*	.211	.000
D6	CER	CTX	CER	-.339	.211	1.000
			OB	-.780*	.211	.003
		HPC	HPC	-.287	.211	1.000
			CTX	-.931*	.211	.000
			CER	-1.270*	.211	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
15min	D1	OB	CER	.490	.211	.218
			HPC	.983*	.211	.000
			CTX	.339	.211	1.000
			STR	1.270*	.211	.000
	OB	HPC	CER	.499	.211	.197
			CTX	-.060	.211	1.000
			STR	.639*	.211	.030
			CER	-.578	.211	.071
	HPC	OB	CER	-.499	.211	.197
			CTX	-.559	.211	.091
			STR	.140	.211	1.000
			CER	-1.077*	.211	.000
	CTX	OB	CER	.060	.211	1.000
			HPC	.559	.211	.091
			STR	.699*	.211	.012
			CER	-.517	.211	.156
	STR	OB	CER	-.639*	.211	.030
			HPC	-.140	.211	1.000
			CTX	-.699*	.211	.012
			CER	-1.217*	.211	.000
	CER	OB	CER	.578	.211	.071
			HPC	1.077*	.211	.000
			CTX	.517	.211	.156
			STR	1.217*	.211	.000
	D2	OB	HPC	.411	.211	.538

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	OB	CTX	CTX	-.192	.211	1.000
			STR	.712*	.211	.010
			CER	-.556	.211	.095
	HPC	OB	OB	-.411	.211	.538
			CTX	-.603	.211	.050
			STR	.301	.211	1.000
	CTX	OB	CER	-.967*	.211	.000
			OB	.192	.211	1.000
			HPC	.603	.211	.050
	STR	OB	STR	.904*	.211	.000
			CER	-.365	.211	.868
			OB	-.712*	.211	.010
D4	OB	HPC	HPC	-.301	.211	1.000
			CTX	-.904*	.211	.000
			CER	-1.268*	.211	.000
	CER	OB	OB	.556	.211	.095
			HPC	.967*	.211	.000
			CTX	.365	.211	.868
	HPC	OB	STR	1.268*	.211	.000
			HPC	.525	.211	.141
			CTX	-.126	.211	1.000
	OB	STR	STR	.828*	.239	.007
			CER	-.382	.200	.581
			OB	-.525	.211	.141
	CTX	OB	CTX	-.651*	.211	.025

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	STR		.302	.239	1.000
			CER	-.908*	.200	.000
		CTX	OB	.126	.211	1.000
			HPC	.651*	.211	.025
			STR	.954*	.239	.001
	STR	CER		-.256	.200	1.000
		STR	OB	-.828*	.239	.007
			HPC	-.302	.239	1.000
		CTX		-.954*	.239	.001
			CER	-1.210*	.230	.000
	HPC	CER	OB	.382	.200	.581
			HPC	.908*	.200	.000
			CTX	.256	.200	1.000
			STR	1.210*	.230	.000
		OB	HPC	.257	.211	1.000
			CTX	-.307	.211	1.000
			STR	.375	.211	.784
			CER	-.623*	.211	.038
		STR	OB	-.257	.211	1.000
			CTX	-.564	.211	.086
			STR	.118	.211	1.000
			CER	-.880*	.211	.001
	CTX	OB		.307	.211	1.000
			HPC	.564	.211	.086
			STR	.681*	.211	.016

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
30min	D1	OB	CER	-.316	.211	1.000
			STR	-.375	.211	.784
			HPC	-.118	.211	1.000
			CTX	-.681*	.211	.016
			CER	-.998*	.211	.000
	D2	HPC	CER	.623*	.211	.038
			OB	.880*	.211	.001
			CTX	.316	.211	1.000
			STR	.998*	.211	.000
			HPC	.512	.211	.167
1hr	D1	OB	CTX	-.182	.211	1.000
			STR	.998*	.211	.000
			CER	-.490	.211	.219
		HPC	OB	-.512	.211	.167
			CTX	-.694*	.211	.013
	D2	HPC	STR	.485	.211	.232
			CER	-1.002*	.211	.000
			OB	.182	.211	1.000
			CTX	.694*	.211	.013
			STR	1.179*	.211	.000
2hr	D1	OB	CER	-.308	.211	1.000
			OB	-.998*	.211	.000
			HPC	-.485	.211	.232
			CTX	-1.179*	.211	.000
			CER	-1.487*	.211	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D2	OB	CER	OB	.490	.211	.219
			HPC	1.002*	.211	.000
			CTX	.308	.211	1.000
			STR	1.487*	.211	.000
	HPC	OB	OB	.539	.211	.119
			CTX	-.164	.211	1.000
			STR	.467	.211	.289
			CER	-.226	.211	1.000
	CTX	OB	OB	-.539	.211	.119
			CTX	-.702*	.211	.011
			STR	-.072	.211	1.000
			CER	-.764*	.211	.004
	STR	OB	OB	.164	.211	1.000
			HPC	.702*	.211	.011
			STR	.630*	.211	.034
			CER	-.062	.211	1.000
	CER	OB	OB	-.467	.211	.289
			HPC	.072	.211	1.000
			CTX	-.630*	.211	.034
			CER	-.692*	.211	.013
	D4	OB	OB	.226	.211	1.000
			HPC	.764*	.211	.004
			CTX	.062	.211	1.000
			STR	.692*	.211	.013
	OB	HPC		.324	.211	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	HPC	CTX	-.224	.211	1.000
			STR	.576	.211	.072
			CER	-.579	.211	.070
	CTX	STR	OB	-.324	.211	1.000
			CTX	-.548	.211	.106
			STR	.252	.211	1.000
	HPC	CER	OB	-.903*	.211	.000
			CTX	.224	.211	1.000
			HPC	.548	.211	.106
	STR	CER	OB	.800*	.211	.002
			STR	-.355	.211	.951
			CTX	-.576	.211	.072
	CER	STR	OB	-.252	.211	1.000
			CTX	-.800*	.211	.002
			CER	-1.155*	.211	.000
D6	OB	CTX	OB	.579	.211	.070
			HPC	.903*	.211	.000
			STR	.355	.211	.951
			CTX	1.155*	.211	.000
	HPC	STR	OB	.592	.211	.058
			CTX	.008	.200	1.000
			STR	.782*	.211	.003
			CER	-.472	.211	.272
	STR	CER	OB	-.592	.211	.058
			CTX	-.584*	.200	.041

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
60min	D1	STR	OB	.190	.211	1.000
			CER	-1.064*	.211	.000
			CTX	-.008	.200	1.000
			HPC	.584*	.200	.041
		STR	OB	.774*	.200	.002
		CER	OB	-.480	.200	.178
		STR	OB	-.782*	.211	.003
			HPC	-.190	.211	1.000
			CTX	-.774*	.200	.002
			CER	-1.254*	.211	.000
	D2	CER	OB	.472	.211	.272
			HPC	1.064*	.211	.000
			CTX	.480	.200	.178
			STR	1.254*	.211	.000
		OB	HPC	.448	.211	.356
	D3	STR	CTX	-.137	.211	1.000
			OB	.762*	.211	.004
			CER	-.432	.211	.430
			OB	-.448	.211	.356
		OB	CTX	-.586	.211	.064
	D4	HPC	STR	.313	.211	1.000
			CER	-.880*	.211	.001
			OB	.137	.211	1.000
			CTX	.586	.211	.064
		OB	STR	.899*	.211	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	CER	CER	-.295	.211	1.000
			STR	-.762*	.211	.004
			HPC	-.313	.211	1.000
			CTX	-.899*	.211	.000
		STR	CER	-1.193*	.211	.000
	Day 2	CER	OB	.432	.211	.430
			HPC	.880*	.211	.001
			CTX	.295	.211	1.000
			STR	1.193*	.211	.000
		OB	HPC	.551	.211	.102
D2	Day 1	HPC	CTX	-.311	.211	1.000
			STR	.756*	.211	.005
			CER	-.543	.211	.113
			OB	-.551	.211	.102
			CTX	-.862*	.211	.001
	Day 2	CTX	STR	.205	.211	1.000
			CER	-1.093*	.211	.000
			OB	.311	.211	1.000
			HPC	.862*	.211	.001
			STR	1.068*	.211	.000
D3	Day 1	STR	CER	-.231	.211	1.000
			OB	-.756*	.211	.005
			HPC	-.205	.211	1.000
			CTX	-1.068*	.211	.000
		STR	CER	-1.299*	.211	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D4	OB	CER	OB	.543	.211	.113
			HPC	1.093*	.211	.000
			CTX	.231	.211	1.000
			STR	1.299*	.211	.000
	HPC	OB	OB	.892*	.211	.000
			CTX	-.094	.211	1.000
			STR	.976*	.211	.000
			CER	-.456	.211	.325
	CTX	OB	OB	-.892*	.211	.000
			CTX	-.985*	.211	.000
			STR	.084	.211	1.000
			CER	-1.348*	.211	.000
	STR	OB	OB	.094	.211	1.000
			HPC	.985*	.211	.000
			STR	1.069*	.211	.000
			CER	-.363	.211	.882
	CER	OB	OB	-.976*	.211	.000
			HPC	-.084	.211	1.000
			CTX	-1.069*	.211	.000
			CER	-1.432*	.211	.000
	D6	OB	OB	.456	.211	.325
			HPC	1.348*	.211	.000
			CTX	.363	.211	.882
			STR	1.432*	.211	.000
		OB	HPC	.474	.211	.264

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
HPC	OB	CTX		-.211	.239	1.000
		STR		.595	.211	.056
		CER		-.460	.211	.312
	CTX	OB		-.474	.211	.264
		STR		-.686*	.239	.048
		CER		.121	.211	1.000
	STR	OB		-.934*	.211	.000
		CTX		.211	.239	1.000
		HPC		.686*	.239	.048
	CER	OB		.807*	.239	.010
		STR		-.249	.239	1.000
		CTX		-.595	.211	.056
	CER	OB		-.121	.211	1.000
		STR		-.807*	.239	.010
		CTX		-.1055*	.211	.000
	STR	OB		.460	.211	.312
		STR		.934*	.211	.000
		CTX		.249	.239	1.000
	CTX	OB		1.055*	.211	.000

Table A.3: The full table of post-hoc pairwise comparisons for Erk1, discussed in §4.4.3. Bonferroni correction for multiple comparisons was used. P-values (“Sig.”) are reported with the standard error (“Std. Error”). Mean differences for which p-values less than .05 are starred for emphasis.

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
pre	D1	OB	HPC	.599	.314	.590
			CTX	1.399*	.314	.000
			STR	1.355*	.314	.000
			CER	.509	.314	1.000
	HPC	OB	OB	-.599	.314	.590
			CTX	.801	.314	.120
			STR	.756	.314	.175
			CER	-.090	.314	1.000
	CTX	OB	OB	-1.399*	.314	.000
			HPC	-.801	.314	.120
			STR	-.044	.314	1.000
			CER	-.890	.314	.053
	STR	OB	OB	-1.355*	.314	.000
			HPC	-.756	.314	.175
			CTX	.044	.314	1.000
			CER	-.846	.314	.080
	CER	OB		-.509	.314	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D2	OB	HPC	HPC	.090	.314	1.000
			CTX	.890	.314	.053
			STR	.846	.314	.080
		HPC	OB	.593	.314	.615
	HPC	CTX	OB	1.685*	.314	.000
		STR	OB	1.528*	.314	.000
		CER	OB	.628	.314	.475
		OB	CTX	-.593	.314	.615
	CTX	OB	CTX	1.092*	.314	.007
		OB	STR	.935*	.314	.035
		OB	CER	.036	.314	1.000
		STR	OB	-1.685*	.314	.000
D4	OB	STR	OB	-1.092*	.314	.007
		STR	OB	-.157	.314	1.000
		STR	CER	-1.056*	.314	.010
		OB	OB	-1.528*	.314	.000
	HPC	OB	OB	-.935*	.314	.035
		OB	CTX	.157	.314	1.000
		OB	CER	-.899*	.314	.049
		OB	OB	-.628	.314	.475
	HPC	OB	HPC	-.036	.314	1.000
		OB	CTX	1.056*	.314	.010
		OB	STR	.899*	.314	.049
		HPC	OB	.404	.257	1.000
	OB	CTX	OB	1.186*	.257	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	STR		1.031*	.257	.001
			CER	.531	.289	.681
		HPC	OB	-.404	.257	1.000
			CTX	.781*	.257	.028
			STR	.627	.257	.158
	CTX	CER		.126	.289	1.000
			OB	-1.186*	.257	.000
		STR	HPC	-.781*	.257	.028
			STR	-.154	.257	1.000
			CER	-.655	.289	.247
	CER	OB		-1.031*	.257	.001
			HPC	-.627	.257	.158
		STR	CTX	.154	.257	1.000
			CER	-.501	.289	.850
			OB	-.531	.289	.681
	D6	HPC		-.126	.289	1.000
			CTX	.655	.289	.247
		OB	STR	.501	.289	.850
			HPC	.417	.314	1.000
			CTX	1.305*	.314	.001
	HPC	STR		1.002	.389	.109
			CER	.550	.314	.825
		OB	OB	-.417	.314	1.000
			CTX	.887	.314	.055
			STR	.585	.389	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
0min	D1	OB	CER	.133	.314	1.000
			CTX	-1.305*	.314	.001
			HPC	-.887	.314	.055
			STR	-.302	.389	1.000
			CER	-.755	.314	.177
	D2	STR	OB	-1.002	.389	.109
			HPC	-.585	.389	1.000
			CTX	.302	.389	1.000
			CER	-.452	.389	1.000
			CER	-.550	.314	.825
	D3	HPC	OB	-.133	.314	1.000
			HPC	.755	.314	.177
			CTX	.452	.389	1.000
			STR	.363	.289	1.000
			STR	1.278*	.289	.000
	D4	CER	OB	.790	.289	.070
			STR	.406	.289	1.000
			OB	-.363	.289	1.000
			CTX	.914*	.257	.005
			STR	.427	.257	.985
	D5	CTX	OB	.042	.257	1.000
			OB	-1.278*	.289	.000
			HPC	-.914*	.257	.005
			STR	-.487	.257	.597
			CER	-.872*	.257	.009

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	STR	OB	-.790	.289	.070
			HPC	-.427	.257	.985
			CTX	.487	.257	.597
			CER	-.385	.257	1.000
	Day 2	CER	OB	-.406	.289	1.000
			HPC	-.042	.257	1.000
			CTX	.872*	.257	.009
			STR	.385	.257	1.000
	Day 3	OB	HPC	.507	.257	.502
			CTX	1.163*	.257	.000
			STR	.882*	.257	.008
			CER	.545	.257	.355
	Day 4	HPC	OB	-.507	.257	.502
			CTX	.656	.257	.117
			STR	.375	.257	1.000
			CER	.038	.257	1.000
	Day 5	CTX	OB	-1.163*	.257	.000
			HPC	-.656	.257	.117
			STR	-.281	.257	1.000
			CER	-.618	.257	.173
	Day 6	STR	OB	-.882*	.257	.008
			HPC	-.375	.257	1.000
			CTX	.281	.257	1.000
			CER	-.337	.257	1.000
	Day 7	CER	OB	-.545	.257	.355

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.	
D4	OB	HPC	HPC	-.038	.257	1.000	
			CTX	.618	.257	.173	
			STR	.337	.257	1.000	
		CER	HPC	.227	.445	1.000	
	HPC		CTX	1.197	.445	.080	
			STR	1.024	.445	.228	
			CER	.487	.445	1.000	
	CTX	OB	OB	-.227	.445	1.000	
		HPC	CTX	.970	.445	.308	
			STR	.797	.445	.753	
			CER	.260	.445	1.000	
	STR	OB	OB	-1.197	.445	.080	
		HPC	HPC	-.970	.445	.308	
			STR	-.173	.445	1.000	
			CER	-.710	.445	1.000	
	CER	OB	OB	-1.024	.445	.228	
		HPC	HPC	-.797	.445	.753	
			CTX	.173	.445	1.000	
			CER	-.537	.445	1.000	
D6	OB	OB	OB	-.487	.445	1.000	
		HPC	HPC	-.260	.445	1.000	
	CTX		CTX	.710	.445	1.000	
			STR	.537	.445	1.000	
	OB	HPC	HPC	.408	.257	1.000	
		CTX	CTX	1.017*	.257	.001	

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
15min	D1	HPC	STR	.820*	.257	.017
			CER	.403	.257	1.000
			OB	-.408	.257	1.000
			CTX	.609	.257	.190
			STR	.412	.257	1.000
	D2	CTX	CER	-.005	.257	1.000
			OB	-1.017*	.257	.001
			HPC	-.609	.257	.190
			STR	-.197	.257	1.000
			CER	-.615	.257	.180
	D3	STR	OB	-.820*	.257	.017
			HPC	-.412	.257	1.000
			CTX	.197	.257	1.000
			CER	-.417	.257	1.000
		CER	OB	-.403	.257	1.000
			HPC	.005	.257	1.000
			CTX	.615	.257	.180
			STR	.417	.257	1.000
			OB	.662	.257	.110
	D4	HPC	CTX	1.416*	.257	.000
			STR	.985*	.257	.002
			CER	.498	.257	.544
			OB	-.662	.257	.110
			CTX	.754*	.257	.039
			STR	.324	.257	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	CTX	CER	-.164	.257	1.000
			OB	-1.416*	.257	.000
			HPC	-.754*	.257	.039
		STR	STR	-.431	.257	.956
			CER	-.918*	.257	.005
	Day 2	STR	OB	-.985*	.257	.002
			HPC	-.324	.257	1.000
			CTX	.431	.257	.956
		CER	STR	-.487	.257	.597
			OB	-.498	.257	.544
D2	Day 1	CER	HPC	.164	.257	1.000
			CTX	.918*	.257	.005
			STR	.487	.257	.597
		OB	HPC	.509	.257	.494
			CTX	1.250*	.257	.000
	Day 2	OB	STR	.900*	.257	.006
			CER	.392	.257	1.000
		HPC	OB	-.509	.257	.494
			CTX	.741*	.257	.045
		STR	STR	.391	.257	1.000
			CER	-.117	.257	1.000
D3	Day 3	CTX	OB	-1.250*	.257	.000
			HPC	-.741*	.257	.045
			STR	-.350	.257	1.000
		CER	STR	-.858*	.257	.011
			CER	-.858*	.257	.011

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D4	OB	STR	OB	-.900*	.257	.006
			HPC	-.391	.257	1.000
			CTX	.350	.257	1.000
			CER	-.508	.257	.498
	CER	CER	OB	-.392	.257	1.000
			HPC	.117	.257	1.000
			CTX	.858*	.257	.011
			STR	.508	.257	.498
	HPC	OB	HPC	.510	.257	.488
			CTX	1.302*	.257	.000
			STR	1.009*	.257	.001
			CER	.537	.241	.274
	CTX	OB	OB	-.510	.257	.488
			CTX	.792*	.257	.025
			STR	.498	.257	.542
			CER	.027	.241	1.000
	STR	OB	OB	-1.302*	.257	.000
			HPC	-.792*	.257	.025
			STR	-.293	.257	1.000
			CER	-.765*	.241	.019
	CER	OB	OB	-1.009*	.257	.001
			HPC	-.498	.257	.542
			CTX	.293	.257	1.000
			CER	-.471	.241	.526

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.	
D6	OB	HPC	HPC	-.027	.241	1.000	
			CTX	.765*	.241	.019	
			STR	.471	.241	.526	
		HPC	OB	.511	.257	.486	
	HPC	CTX	OB	1.168*	.257	.000	
		STR	OB	.948*	.257	.003	
		CER	OB	.439	.257	.898	
		OB	CTX	-.511	.257	.486	
	CTX	OB	CTX	.657	.257	.116	
		OB	STR	.437	.257	.909	
		OB	CER	-.072	.257	1.000	
		OB	OB	-1.168*	.257	.000	
30min	D1	HPC	OB	-.657	.257	.116	
			STR	-.220	.257	1.000	
			CER	-.729	.257	.052	
		STR	OB	-.948*	.257	.003	
	OB		OB	-.437	.257	.909	
			CTX	.220	.257	1.000	
			CER	-.509	.257	.493	
	CER	OB	OB	-.439	.257	.898	
			OB	.072	.257	1.000	
			CTX	.729	.257	.052	
			STR	.509	.257	.493	
	OB	HPC	OB	.513	.257	.476	
		CTX	OB	1.102*	.257	.000	

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
HPC	STR	STR		.830*	.257	.015
		CER		.430	.257	.961
	OB	OB		-.513	.257	.476
		CTX		.589	.257	.233
		STR		.317	.257	1.000
		CER		-.083	.257	1.000
		CTX		-1.102*	.257	.000
	HPC	OB		-.589	.257	.233
		STR		-.272	.257	1.000
		CER		-.672	.257	.099
		STR		-.830*	.257	.015
CER	OB	OB		-.317	.257	1.000
		CTX		.272	.257	1.000
		CER		-.400	.257	1.000
		STR		-.430	.257	.961
	HPC	OB		.083	.257	1.000
		CTX		.672	.257	.099
		STR		.400	.257	1.000
		STR		.594	.257	.222
	D2	OB		1.336*	.257	.000
		CTX		1.012*	.257	.001
		STR		.442	.257	.874
		CER		-.594	.257	.222
STR	HPC	OB		.742*	.257	.045
		CTX		.418	.257	1.000
		STR				

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D4	OB	CER	CER	-.152	.257	1.000
			CTX	-1.336*	.257	.000
			HPC	-.742*	.257	.045
			STR	-.324	.257	1.000
	STR	HPC	CER	-.894*	.257	.007
			OB	-1.012*	.257	.001
			CTX	-.418	.257	1.000
			STR	.324	.257	1.000
	CER	STR	CER	-.570	.257	.281
			OB	-.442	.257	.874
			HPC	.152	.257	1.000
			CTX	.894*	.257	.007
	OB	D4	STR	.570	.257	.281
			HPC	.394	.257	1.000
			CTX	.994*	.257	.002
			STR	.824*	.257	.016
	HPC	OB	CER	.338	.257	1.000
			OB	-.394	.257	1.000
			CTX	.600	.257	.208
			STR	.430	.257	.961
	CTX	HPC	CER	-.056	.257	1.000
			OB	-.994*	.257	.002
			HPC	-.600	.257	.208
			STR	-.170	.257	1.000
	CER	STR	CER	-.656	.257	.117

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	STR	OB	-.824*	.257	.016
			HPC	-.430	.257	.961
			CTX	.170	.257	1.000
			CER	-.486	.257	.605
	CER	CER	OB	-.338	.257	1.000
			HPC	.056	.257	1.000
			CTX	.656	.257	.117
			STR	.486	.257	.605
	HPC	OB	HPC	.349	.257	1.000
			CTX	.891*	.241	.003
			STR	.807*	.257	.020
			CER	.355	.257	1.000
	CTX	OB	OB	-.349	.257	1.000
			CTX	.542	.241	.261
			STR	.457	.257	.769
			CER	.006	.257	1.000
	STR	OB	OB	-.891*	.241	.003
			HPC	-.542	.241	.261
			STR	-.085	.241	1.000
			CER	-.536	.241	.278
	CER	OB	OB	-.807*	.257	.020
			HPC	-.457	.257	.769
			CTX	.085	.241	1.000
			CER	-.451	.257	.809

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
60min	D1	OB	HPC	-.006	.257	1.000
			CTX	.536	.241	.278
			STR	.451	.257	.809
			HPC	.446	.257	.843
			CTX	1.109*	.257	.000
			STR	.769*	.257	.033
			CER	.280	.257	1.000
			HPC	-.446	.257	.843
			CTX	.663	.257	.109
			STR	.322	.257	1.000
			CER	-.166	.257	1.000
			CTX	OB	-1.109*	.257
D2			HPC	-.663	.257	.109
			STR	-.340	.257	1.000
			CER	-.829*	.257	.016
			STR	OB	-.769*	.257
			HPC	-.322	.257	1.000
			CTX	.340	.257	1.000
			CER	-.488	.257	.592
			CER	OB	-.280	.257
			HPC	.166	.257	1.000
			CTX	.829*	.257	.016
			STR	.488	.257	.592
			OB	HPC	.502	.257
			CTX	1.276*	.257	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	HPC	STR		.885*	.257	.007
			CER	.558	.257	.314
		OB		-.502	.257	.527
			CTX	.774*	.257	.031
			STR	.384	.257	1.000
	CTX	CER		.056	.257	1.000
			OB	-1.276*	.257	.000
		STR		-.774*	.257	.031
			STR	-.390	.257	1.000
			CER	-.718	.257	.059
D2	STR	OB		-.885*	.257	.007
			HPC	-.384	.257	1.000
		CTX		.390	.257	1.000
			CER	-.327	.257	1.000
			OB	-.558	.257	.314
	CER	HPC		-.056	.257	1.000
			CTX	.718	.257	.059
		STR		.327	.257	1.000
			OB	-.402	.257	1.000
			CTX	1.126*	.257	.000
D3	D4	STR		1.012*	.257	.001
			CER	.512	.257	.482
		HPC		-.402	.257	1.000
			OB	.725	.257	.055
			CTX	.610	.257	.188

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	Day 1	CER	CER	.110	.257	1.000
			CTX	-1.126*	.257	.000
			HPC	-.725	.257	.055
		STR	STR	-.114	.257	1.000
	Day 2	CER	CER	-.615	.257	.180
			STR	-1.012*	.257	.001
			HPC	-.610	.257	.188
		CTX	CTX	.114	.257	1.000
	Day 3	CER	CER	-.500	.257	.533
			OB	-.512	.257	.482
			HPC	-.110	.257	1.000
		STR	CTX	.615	.257	.180
	Day 4	CER	STR	.500	.257	.533
			OB	-.425	.257	1.000
			CTX	1.270*	.289	.000
		STR	STR	.915*	.257	.005
D7	Day 5	CER	CER	.423	.257	1.000
		HPC	OB	-.425	.257	1.000
			CTX	.845*	.289	.040
		STR	STR	.491	.257	.580
	Day 6	CER	CER	-.002	.257	1.000
			OB	-1.270*	.289	.000
			CTX	-.845*	.289	.040
		STR	STR	-.354	.289	1.000
	Day 7	CER	CER	-.847*	.289	.039
			OB	-.425	.289	1.000
			CTX	-.845*	.289	.040
		STR	STR	-.354	.289	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
CER	Day 1	STR	OB	-.915*	.257	.005
			HPC	-.491	.257	.580
			CTX	.354	.289	1.000
			CER	-.493	.257	.570
	Day 4	CER	OB	-.423	.257	1.000
			HPC	.002	.257	1.000
			CTX	.847*	.289	.039
			STR	.493	.257	.570

Table A.4: The full table of post-hoc pairwise comparisons for Erk2 mRNA, discussed in §4.4.3. Bonferroni correction for multiple comparisons was used. P-values (“Sig.”) are reported with the standard error (“Std. Error”). Mean differences for which p-values less than .05 are starred for emphasis.

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
pre	D1	OB	HPC	.400	.156	.113
			CTX	.464*	.156	.034
			STR	.284	.156	.708
			CER	.805*	.156	.000
	D4	HPC	OB	-.400	.156	.113
			CTX	.064	.156	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	STR		-.116	.156	1.000
			CER	.405	.156	.104
		CTX	OB	-.464*	.156	.034
			HPC	-.064	.156	1.000
		STR		-.180	.156	1.000
			CER	.341	.156	.304
		STR	OB	-.284	.156	.708
			HPC	.116	.156	1.000
		CTX		.180	.156	1.000
			CER	.521*	.156	.011
	Day 2	CER	OB	-.805*	.156	.000
			HPC	-.405	.156	.104
		CTX		-.341	.156	.304
			STR	-.521*	.156	.011
		D2	OB	.431	.156	.065
			CTX	.721*	.156	.000
	D2	STR		.447*	.156	.048
			CER	1.023*	.156	.000
		HPC	OB	-.431	.156	.065
			CTX	.290	.156	.655
		STR		.016	.156	1.000
			CER	.592*	.156	.002
	D3	CTX	OB	-.721*	.156	.000
			HPC	-.290	.156	.655
		STR		-.274	.156	.816

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D4	OB	CER		.302	.156	.550
		STR	OB	-.447*	.156	.048
			HPC	-.016	.156	1.000
			CTX	.274	.156	.816
		CER		.576*	.156	.003
	CER	OB		-1.023*	.156	.000
			HPC	-.592*	.156	.002
			CTX	-.302	.156	.550
			STR	-.576*	.156	.003
		OB	HPC	.312	.128	.157
D4	HPC		CTX	.624*	.128	.000
			STR	.293	.128	.227
		CER		.915*	.143	.000
		OB		-.312	.128	.157
			CTX	.312	.128	.155
	CTX		STR	-.018	.128	1.000
		CER		.603*	.143	.000
		OB		-.624*	.128	.000
			HPC	-.312	.128	.155
			STR	-.330	.128	.105
STR	CER		CER	.291	.143	.429
		OB		-.293	.128	.227
			HPC	.018	.128	1.000
			CTX	.330	.128	.105
			CER	.621*	.143	.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	CER	OB	-.915*	.143	.000
			HPC	-.603*	.143	.000
			CTX	-.291	.143	.429
			STR	-.621*	.143	.000
	HPC	OB	OB	.324	.156	.399
			CTX	.633*	.156	.001
			STR	.393	.191	.418
			CER	.932*	.156	.000
	CTX	OB	OB	-.324	.156	.399
			CTX	.309	.156	.496
			STR	.069	.191	1.000
			CER	.608*	.156	.001
	STR	OB	OB	-.633*	.156	.001
			HPC	-.309	.156	.496
			STR	-.240	.191	1.000
			CER	.299	.156	.576
	CER	OB	OB	-.393	.191	.418
			HPC	-.069	.191	1.000
			CTX	.240	.191	1.000
			CER	.539	.191	.054
	0min	OB	OB	-.932*	.156	.000
			HPC	-.608*	.156	.001
			CTX	-.299	.156	.576
			STR	-.539	.191	.054
D1		OB	HPC	.223	.143	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	HPC	CTX	OB	.533*	.143	.003
			STR	.174	.143	1.000
			CER	.768*	.143	.000
		OB	OB	-.223	.143	1.000
	CTX	CTX	OB	.310	.128	.161
		STR	OB	-.049	.128	1.000
		CER	OB	.545*	.128	.000
	STR	OB	OB	-.533*	.143	.003
		STR	OB	-.174	.143	1.000
		STR	HPC	.049	.128	1.000
		CER	OB	.359	.128	.055
D2	CER	OB	OB	.234	.128	.680
		STR	OB	-.768*	.143	.000
		STR	HPC	-.545*	.128	.000
		CER	OB	-.234	.128	.680
	D2	OB	OB	-.593*	.128	.000
		OB	HPC	.363	.128	.050
		OB	CTX	.486*	.128	.002
	HPC	OB	STR	.130	.128	1.000
		OB	CER	.844*	.128	.000
		CTX	OB	-.363	.128	.050
		CTX	CTX	.123	.128	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	STR	STR	-.233	.128	.696
			CER	.481*	.128	.002
		CTX	OB	-.486*	.128	.002
			HPC	-.123	.128	1.000
		STR	STR	-.356	.128	.059
			CER	.358	.128	.056
		STR	OB	-.130	.128	1.000
			HPC	.233	.128	.696
			CTX	.356	.128	.059
			CER	.714*	.128	.000
			CER	-.844*	.128	.000
D4	Day 4	HPC	OB	-.481*	.128	.002
			CTX	-.358	.128	.056
		STR	STR	-.714*	.128	.000
			OB	.185	.221	1.000
		CTX	CTX	.637*	.221	.045
			STR	.456	.221	.407
		HPC	CER	.872*	.221	.001
			OB	-.185	.221	1.000
			CTX	.452	.221	.425
			STR	.270	.221	1.000
			CER	.687*	.221	.022
	Day 7	CTX	OB	-.637*	.221	.045
			HPC	-.452	.221	.425
		STR	STR	-.181	.221	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	CER	CER	.235	.221	1.000
			STR	-.456	.221	.407
			HPC	-.270	.221	1.000
			CTX	.181	.221	1.000
	CER	OB	CER	.417	.221	.612
			STR	-.872*	.221	.001
			HPC	-.687*	.221	.022
			CTX	-.235	.221	1.000
	HPC	OB	STR	-.417	.221	.612
			OB	.226	.128	.781
			CTX	.385*	.128	.030
			STR	.248	.128	.537
D6	CER	OB	CER	.692*	.128	.000
			STR	-.226	.128	.781
			HPC	.158	.128	1.000
			CTX	.022	.128	1.000
	CTX	OB	CER	.466*	.128	.003
			STR	-.385*	.128	.030
			HPC	-.158	.128	1.000
			STR	-.137	.128	1.000
	STR	OB	CER	.308	.128	.169
			OB	-.248	.128	.537
			HPC	-.022	.128	1.000
			CTX	.137	.128	1.000
	CER	OB	CER	.445*	.128	.006

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
15min	D1	OB	CER	.692*	.128	.000
			HPC	-.466*	.128	.003
			CTX	-.308	.128	.169
			STR	-.445*	.128	.006
	OB	HPC	OB	.405*	.128	.018
			CTX	.520*	.128	.001
			STR	.313	.128	.151
			CER	.798*	.128	.000
	HPC	OB	OB	-.405*	.128	.018
			CTX	.115	.128	1.000
			STR	-.092	.128	1.000
			CER	.394*	.128	.024
	CTX	OB	OB	-.520*	.128	.001
			HPC	-.115	.128	1.000
			STR	-.206	.128	1.000
			CER	.279	.128	.302
	STR	OB	OB	-.313	.128	.151
			HPC	.092	.128	1.000
			CTX	.206	.128	1.000
			CER	.485*	.128	.002
	CER	OB	OB	-.798*	.128	.000
			HPC	-.394*	.128	.024
			CTX	-.279	.128	.302
			STR	-.485*	.128	.002
	D2	OB	HPC	.279	.128	.301

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	OB	CTX	CTX	.477*	.128	.003
			STR	.139	.128	1.000
			CER	.751*	.128	.000
	HPC	OB	OB	-.279	.128	.301
			CTX	.198	.128	1.000
			STR	-.140	.128	1.000
	CTX	CER	CER	.472*	.128	.003
			OB	-.477*	.128	.003
			HPC	-.198	.128	1.000
	STR	STR	STR	-.338	.128	.089
			CER	.274	.128	.333
			OB	-.139	.128	1.000
D4	OB	HPC	HPC	.140	.128	1.000
			CTX	.338	.128	.089
			CER	.612*	.128	.000
	CER	CER	OB	-.751*	.128	.000
			HPC	-.472*	.128	.003
			CTX	-.274	.128	.333
	HPC	STR	STR	-.612*	.128	.000
			OB	.303	.128	.188
			CTX	.495*	.128	.002
	STR	CER	STR	.261	.128	.426
			CER	.787*	.119	.000
			OB	-.303	.128	.188
	HPC	CTX	CTX	.192	.128	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	STR	STR	-.042	.128	1.000
			CER	.484*	.119	.001
		CTX	OB	-.495*	.128	.002
			HPC	-.192	.128	1.000
		STR	STR	-.234	.128	.686
			CER	.292	.119	.154
	HPC	STR	OB	-.261	.128	.426
			HPC	.042	.128	1.000
		CTX	CTX	.234	.128	.686
			CER	.526*	.119	.000
		CER	OB	-.787*	.119	.000
			HPC	-.484*	.119	.001
	STR	CTX	CTX	-.292	.119	.154
			STR	-.526*	.119	.000
		OB	HPC	.325	.128	.117
			CTX	.633*	.128	.000
		CTX	STR	.280	.128	.299
			CER	.870*	.128	.000
	HPC	OB	OB	-.325	.128	.117
			CTX	.308	.128	.170
		STR	STR	-.046	.128	1.000
			CER	.545*	.128	.000
		STR	OB	-.633*	.128	.000
			HPC	-.308	.128	.170
			STR	-.353	.128	.063

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
30min	D1	OB	CER	.237	.128	.647
			STR	-.280	.128	.299
			HPC	.046	.128	1.000
			CTX	.353	.128	.063
			CER	.591*	.128	.000
	D2	HPC	CER	-.870*	.128	.000
			OB	-.545*	.128	.000
			HPC	-.237	.128	.647
			CTX	-.591*	.128	.000
			STR	.276	.128	.318
1hr	D1	OB	CTX	.406*	.128	.017
			STR	.306	.128	.176
			CER	.702*	.128	.000
		HPC	OB	-.276	.128	.318
			CTX	.130	.128	1.000
	D2	HPC	STR	.030	.128	1.000
			CER	.426*	.128	.010
			OB	-.406*	.128	.017
			CTX	-.130	.128	1.000
			STR	-.100	.128	1.000
24hr	D1	OB	CER	.296	.128	.216
			OB	-.306	.128	.176
			HPC	-.030	.128	1.000
			CTX	.100	.128	1.000
			CER	.396*	.128	.022

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D2	OB	CER	OB	-.702*	.128	.000
			HPC	-.426*	.128	.010
			CTX	-.296	.128	.216
			STR	-.396*	.128	.022
	HPC	OB	OB	.365*	.128	.048
			CTX	.551*	.128	.000
			STR	.124	.128	1.000
			CER	.844*	.128	.000
	CTX	OB	OB	-.365*	.128	.048
			CTX	.186	.128	1.000
			STR	-.241	.128	.610
			CER	.479*	.128	.002
	STR	OB	OB	-.551*	.128	.000
			HPC	-.186	.128	1.000
			STR	-.427*	.128	.010
			CER	.293	.128	.229
	CER	OB	OB	-.124	.128	1.000
			HPC	.241	.128	.610
			CTX	.427*	.128	.010
			CER	.720*	.128	.000
	D4	OB	OB	-.844*	.128	.000
			HPC	-.479*	.128	.002
			CTX	-.293	.128	.229
			STR	-.720*	.128	.000
	OB	OB	HPC	.221	.128	.847

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D6	OB	HPC	CTX	.452*	.128	.005
			STR	.133	.128	1.000
			CER	.724*	.128	.000
		CTX	OB	-.221	.128	.847
	STR	CTX	OB	.230	.128	.728
		STR	OB	-.088	.128	1.000
		CER	OB	.502*	.128	.001
	CER	CTX	OB	-.452*	.128	.005
		STR	OB	-.230	.128	.728
		STR	OB	-.318	.128	.136
		CER	OB	.272	.128	.346
	D6	STR	OB	-.133	.128	1.000
		STR	OB	.088	.128	1.000
		CTX	OB	.318	.128	.136
		CER	OB	.590*	.128	.000
	HPC	CER	OB	-.724*	.128	.000
		CER	OB	-.502*	.128	.001
		CTX	OB	-.272	.128	.346
		STR	OB	-.590*	.128	.000
	OB	OB	HPC	.232	.128	.712
		OB	CTX	.514*	.119	.000
		OB	STR	.228	.128	.760
		OB	CER	.712*	.128	.000
	CTX	HPC	OB	-.232	.128	.712
		HPC	OB	.282	.119	.193

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
60min	D1	OB	STR	-.004	.128	1.000
			CER	.481*	.128	.002
			CTX	-.514*	.119	.000
			HPC	-.282	.119	.193
			STR	-.286	.119	.178
	D2	HPC	CER	.199	.119	.983
			STR	-.228	.128	.760
			CTX	.004	.128	1.000
			CER	.286	.119	.178
			OB	.484*	.128	.002
120min	D1	OB	CER	-.712*	.128	.000
			STR	-.481*	.128	.002
			CTX	-.199	.119	.983
			STR	-.484*	.128	.002
			HPC	.234	.128	.681
	D2	HPC	CTX	.385*	.128	.030
			STR	.249	.128	.528
			CER	.665*	.128	.000
			OB	-.234	.128	.681
			CTX	.150	.128	1.000
240min	D1	OB	STR	.015	.128	1.000
			CER	.430*	.128	.009
			CTX	-.385*	.128	.030
	D2	HPC	OB	-.150	.128	1.000
			STR	-.136	.128	1.000

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	CER	CER	.280	.128	.296
			STR	-.249	.128	.528
			HPC	-.015	.128	1.000
			CTX	.136	.128	1.000
		CER		.416*	.128	.014
	Day 2	CER	OB	-.665*	.128	.000
			HPC	-.430*	.128	.009
			CTX	-.280	.128	.296
			STR	-.416*	.128	.014
		OB	HPC	.294	.128	.226
D2	Day 1	HPC	CTX	.432*	.128	.009
			STR	.266	.128	.387
			CER	.829*	.128	.000
			OB	-.294	.128	.226
			CTX	.138	.128	1.000
	Day 2	CTX	STR	-.028	.128	1.000
			CER	.535*	.128	.000
			OB	-.432*	.128	.009
			HPC	-.138	.128	1.000
			STR	-.166	.128	1.000
D3	Day 1	STR	CER	.397*	.128	.022
			OB	-.266	.128	.387
			HPC	.028	.128	1.000
			CTX	.166	.128	1.000
		OB	CER	.563*	.128	.000
	Day 2	CER	CER			

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
D4	OB	CER	OB	-.829*	.128	.000
			HPC	-.535*	.128	.000
			CTX	-.397*	.128	.022
			STR	-.563*	.128	.000
	HPC	OB	OB	.283	.128	.278
			CTX	.461*	.128	.004
			STR	.257	.128	.454
			CER	.855*	.128	.000
	CTX	OB	OB	-.283	.128	.278
			CTX	.178	.128	1.000
			STR	-.026	.128	1.000
			CER	.571*	.128	.000
	STR	OB	OB	-.461*	.128	.004
			HPC	-.178	.128	1.000
			STR	-.204	.128	1.000
			CER	.393*	.128	.024
	CER	OB	OB	-.257	.128	.454
			HPC	.026	.128	1.000
			CTX	.204	.128	1.000
			CER	.597*	.128	.000
	D6	OB	OB	-.855*	.128	.000
			HPC	-.571*	.128	.000
			CTX	-.393*	.128	.024
			STR	-.597*	.128	.000
	OB	HPC		.337	.128	.091

Delay	Day	(A) Region	(B) Region	(A-B) Mean Difference	Std. Error	Sig.
HPC	OB	CTX		.652*	.143	.000
		STR		.363*	.128	.049
		CER		.897*	.128	.000
	CTX	OB		-.337	.128	.091
		STR		.315	.143	.288
		CER		.027	.128	1.000
	STR	OB		.560*	.128	.000
		CTX		-.652*	.143	.000
		HPC		-.315	.143	.288
	CER	OB		-.288	.143	.451
		STR		.245	.143	.874
		CTX		-.363*	.128	.049
	CER	OB		-.027	.128	1.000
		STR		.288	.143	.451
		CTX		.534*	.128	.000
	STR	OB		-.897*	.128	.000
		CTX		-.560*	.128	.000
		STR		-.245	.143	.874
	CTX	OB		-.534*	.128	.000

Table A.5: The full table of post-hoc pairwise comparisons for Arc mRNA, discussed in §4.4.4. Bonferroni correction for multiple comparisons was used. P-values (“Sig.”) are reported with the standard error (“Std. Error”). Mean differences for which p-values less than .05 are starred for emphasis.

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
OB	D1	pre	0min	-1.789*	.357	.000
			15min	-1.690*	.357	.000
			30min	-1.270*	.357	.005
			60min	-1.006	.357	.055
	0min	pre	0min	1.789*	.357	.000
			15min	.098	.320	1.000
			30min	.518	.320	1.000
			60min	.783	.320	.154
	15min	pre	15min	1.690*	.357	.000
			0min	-.098	.320	1.000
			30min	.420	.320	1.000
			60min	.684	.320	.338
	30min	pre	30min	1.270*	.357	.005
			0min	-.518	.320	1.000
			15min	-.420	.320	1.000
			60min	.264	.320	1.000
	60min	pre		1.006	.357	.055

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D2	pre	0min		-.783	.320	.154
			15min	-.684	.320	.338
			30min	-.264	.320	1.000
			60min	-1.115*	.357	.021
	0min	pre	15min	-.415	.357	1.000
			30min	-.528	.357	1.000
			60min	.265	.357	1.000
				1.115*	.357	.021
	15min	pre	15min	.699	.320	.301
			30min	.586	.320	.685
			60min	1.380*	.320	.000
				.415	.357	1.000
D4	30min	pre	0min	-.699	.320	.301
			30min	-.113	.320	1.000
			60min	.680	.320	.348
				.528	.357	1.000
	60min	pre	0min	-.586	.320	.685
			15min	.113	.320	1.000
			60min	.794	.320	.140
				-.265	.357	1.000
	15min	pre	0min	-1.380*	.320	.000
			15min	-.680	.320	.348
			30min	-.794	.320	.140
				-.547	.452	1.000
	D4	pre	15min	-1.537*	.319	.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			30min	-.790	.320	.145
			60min	.044	.320	1.000
		0min	pre	.547	.452	1.000
			15min	-.990	.452	.298
			30min	-.243	.452	1.000
			60min	.590	.452	1.000
		15min	pre	1.537*	.319	.000
			0min	.990	.452	.298
			30min	.747	.320	.205
			60min	1.581*	.320	.000
		30min	pre	.790	.320	.145
			0min	.243	.452	1.000
			15min	-.747	.320	.205
			60min	.833	.320	.100
		60min	pre	-.044	.320	1.000
			0min	-.590	.452	1.000
			15min	-1.581*	.320	.000
			30min	-.833	.320	.100
D6	pre	0min		-.564	.357	1.000
			15min	-1.065*	.357	.033
			30min	-.937	.357	.095
			60min	-.619	.357	.852
		0min	pre	.564	.357	1.000
			15min	-.501	.320	1.000
			30min	-.373	.320	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			60min	-.055	.320	1.000
		15min	pre	1.065*	.357	.033
			0min	.501	.320	1.000
			30min	.128	.320	1.000
			60min	.446	.320	1.000
		30min	pre	.937	.357	.095
			0min	.373	.320	1.000
			15min	-.128	.320	1.000
			60min	.319	.319	1.000
		60min	pre	.619	.357	.852
			0min	.055	.320	1.000
			15min	-.446	.320	1.000
			30min	-.319	.319	1.000
HPC	D1	pre	0min	-1.058*	.357	.035
			15min	-1.341*	.357	.002
			30min	-1.239*	.357	.007
			60min	-1.029*	.357	.045
		0min	pre	1.058*	.357	.035
			15min	-.283	.320	1.000
			30min	-.181	.320	1.000
			60min	.029	.320	1.000
		15min	pre	1.341*	.357	.002
			0min	.283	.320	1.000
			30min	.102	.320	1.000
			60min	.312	.320	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	30min	pre	1.239*	.357	.007
			0min	.181	.320	1.000
			15min	-.102	.320	1.000
			60min	.210	.320	1.000
	Day 2	60min	pre	1.029*	.357	.045
			0min	-.029	.320	1.000
			15min	-.312	.320	1.000
			30min	-.210	.320	1.000
	Day 3	D2	0min	.025	.357	1.000
			15min	-.918	.357	.111
			30min	-.633	.357	.783
			60min	-.682	.357	.582
	Day 4	0min	pre	-.025	.357	1.000
			15min	-.942*	.320	.037
			30min	-.658	.320	.412
			60min	-.706	.320	.285
	Day 5	15min	pre	.918	.357	.111
			0min	.942*	.320	.037
			30min	.284	.320	1.000
			60min	.236	.320	1.000
	Day 6	30min	pre	.633	.357	.783
			0min	.658	.320	.412
			15min	-.284	.320	1.000
			60min	-.048	.320	1.000
	Day 7	60min	pre	.682	.357	.582

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D4	pre	0min		.706	.320	.285
			15min	-.236	.320	1.000
			30min	.048	.320	1.000
			60min	-1.044	.452	.221
	0min	15min		-1.069*	.319	.010
			30min	-1.132*	.320	.005
			60min	-.962*	.320	.030
			pre	1.044	.452	.221
	15min	15min		-.025	.452	1.000
			30min	-.087	.452	1.000
			60min	.082	.452	1.000
			pre	1.069*	.319	.010
D6	30min	0min		.025	.452	1.000
			30min	-.062	.320	1.000
			60min	.107	.320	1.000
			pre	1.132*	.320	.005
	60min	0min		.087	.452	1.000
			15min	.062	.320	1.000
			60min	.169	.320	1.000
			pre	.962*	.320	.030
	D6	15min		-.082	.452	1.000
			15min	-.107	.320	1.000
			30min	-.169	.320	1.000
			pre	-.934	.357	.098
	pre	15min		-1.289*	.357	.004

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			30min	-.855	.357	.178
			60min	-1.113*	.357	.022
		0min	pre	.934	.357	.098
			15min	-.354	.320	1.000
			30min	.079	.320	1.000
			60min	-.178	.320	1.000
		15min	pre	1.289*	.357	.004
			0min	.354	.320	1.000
			30min	.434	.320	1.000
			60min	.176	.320	1.000
		30min	pre	.855	.357	.178
			0min	-.079	.320	1.000
			15min	-.434	.320	1.000
			60min	-.257	.319	1.000
		60min	pre	1.113*	.357	.022
			0min	.178	.320	1.000
			15min	-.176	.320	1.000
			30min	.257	.319	1.000
CTX	D1	pre	0min	-1.646*	.357	.000
			15min	-2.380*	.357	.000
			30min	-2.308*	.357	.000
			60min	-2.090*	.357	.000
		0min	pre	1.646*	.357	.000
			15min	-.734	.320	.229
			30min	-.663	.320	.398

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
		60min		-.444	.320	1.000
	15min	pre		2.380*	.357	.000
		0min		.734	.320	.229
		30min		.071	.320	1.000
		60min		.289	.320	1.000
	30min	pre		2.308*	.357	.000
		0min		.663	.320	.398
		15min		-.071	.320	1.000
		60min		.218	.320	1.000
	60min	pre		2.090*	.357	.000
		0min		.444	.320	1.000
		15min		-.289	.320	1.000
		30min		-.218	.320	1.000
D2	pre	0min		-.976	.357	.070
		15min		-1.623*	.357	.000
		30min		-1.389*	.357	.001
		60min		-1.529*	.357	.000
	0min	pre		.976	.357	.070
		15min		-.647	.320	.446
		30min		-.413	.320	1.000
		60min		-.554	.320	.853
	15min	pre		1.623*	.357	.000
		0min		.647	.320	.446
		30min		.234	.320	1.000
		60min		.093	.320	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D4	0min	30min	pre	1.389*	.357	.001
		0min		.413	.320	1.000
		15min		-.234	.320	1.000
		60min		-.141	.320	1.000
	60min	60min	pre	1.529*	.357	.000
		0min		.554	.320	.853
		15min		-.093	.320	1.000
		30min		.141	.320	1.000
	D4	0min	pre	-1.240	.452	.067
		15min		-1.503*	.356	.000
		30min		-1.184*	.320	.003
		60min		-1.098*	.320	.007
D4	0min	0min	pre	1.240	.452	.067
		15min		-.263	.479	1.000
		30min		.056	.452	1.000
		60min		.142	.452	1.000
	15min	15min	pre	1.503*	.356	.000
		0min		.263	.479	1.000
		30min		.320	.356	1.000
		60min		.405	.356	1.000
	30min	30min	pre	1.184*	.320	.003
		0min		-.056	.452	1.000
		15min		-.320	.356	1.000
		60min		.085	.320	1.000
	60min	60min	pre	1.098*	.320	.007

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D6	pre	0min		-.142	.452	1.000
			15min	-.405	.356	1.000
			30min	-.085	.320	1.000
			60min	-1.568*	.357	.000
	0min	pre	15min	-1.195*	.357	.010
			30min	-1.654*	.339	.000
			60min	-1.419*	.391	.004
				1.568*	.357	.000
	15min	pre	15min	.373	.320	1.000
			30min	-.086	.299	1.000
			60min	.149	.357	1.000
				1.195*	.357	.010
STR	D1	0min	0min	-.373	.320	1.000
			30min	-.459	.299	1.000
			60min	-.224	.357	1.000
				1.654*	.339	.000
	30min	pre	0min	.086	.299	1.000
			15min	.459	.299	1.000
			60min	.236	.337	1.000
				1.419*	.391	.004
	60min	pre	0min	-.149	.357	1.000
			15min	.224	.357	1.000
			30min	-.236	.337	1.000
				-1.656*	.357	.000
	STR	D1	15min	-1.967*	.357	.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
		30min		-1.813*	.357	.000
		60min		-1.732*	.357	.000
0min		pre		1.656*	.357	.000
		15min		-.311	.320	1.000
		30min		-.157	.320	1.000
		60min		-.076	.320	1.000
15min		pre		1.967*	.357	.000
		0min		.311	.320	1.000
		30min		.154	.320	1.000
		60min		.235	.320	1.000
30min		pre		1.813*	.357	.000
		0min		.157	.320	1.000
		15min		-.154	.320	1.000
		60min		.081	.320	1.000
60min		pre		1.732*	.357	.000
		0min		.076	.320	1.000
		15min		-.235	.320	1.000
		30min		-.081	.320	1.000
D2	pre	0min		-1.026*	.357	.046
		15min		-1.138*	.357	.017
		30min		-.920	.357	.110
		60min		-1.104*	.357	.024
0min		pre		1.026*	.357	.046
		15min		-.111	.320	1.000
		30min		.107	.320	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	15min	60min	-.078	.320	1.000
			pre	1.138*	.357	.017
		Day 2	0min	.111	.320	1.000
			30min	.218	.320	1.000
			60min	.033	.320	1.000
	Day 3	30min	pre	.920	.357	.110
			0min	-.107	.320	1.000
		Day 4	15min	-.218	.320	1.000
			60min	-.184	.320	1.000
			pre	1.104*	.357	.024
D2	Day 1	60min	0min	.078	.320	1.000
			15min	-.033	.320	1.000
		Day 2	30min	.184	.320	1.000
			0min	-.770	.452	.902
			15min	-1.173*	.319	.003
	Day 3	30min	30min	-.849	.320	.087
			60min	-.616	.320	.556
		Day 4	0min	.770	.452	.902
			15min	-.402	.452	1.000
			30min	-.078	.452	1.000
D3	Day 1	60min	60min	.155	.452	1.000
			pre	1.173*	.319	.003
		Day 2	0min	.402	.452	1.000
			30min	.324	.320	1.000
			60min	.557	.320	.832

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D6	30min	30min	pre	.849	.320	.087
		0min		.078	.452	1.000
		15min		-.324	.320	1.000
		60min		.233	.320	1.000
	60min	60min	pre	.616	.320	.556
		0min		-.155	.452	1.000
		15min		-.557	.320	.832
		30min		-.233	.320	1.000
	0min	pre	0min	-1.229	.450	.070
			15min	-1.420*	.450	.019
			30min	-1.281*	.450	.049
			60min	-1.198	.450	.085
	15min	0min	pre	1.229	.450	.070
			15min	-.191	.320	1.000
			30min	-.052	.320	1.000
			60min	.030	.320	1.000
	30min	15min	pre	1.420*	.450	.019
			0min	.191	.320	1.000
			30min	.139	.320	1.000
			60min	.222	.320	1.000
	60min	30min	pre	1.281*	.450	.049
			0min	.052	.320	1.000
			15min	-.139	.320	1.000
			60min	.083	.319	1.000
	60min	60min	pre	1.198	.450	.085

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
CER	D1	pre	0min	-.030	.320	1.000
			15min	-.222	.320	1.000
			30min	-.083	.319	1.000
			60min	.129	.357	1.000
	0min	pre	15min	.023	.357	1.000
			30min	-.037	.357	1.000
			60min	-.199	.357	1.000
			15min	-.129	.357	1.000
	15min	pre	0min	-.106	.320	1.000
			30min	-.166	.320	1.000
			60min	-.328	.320	1.000
			30min	-.023	.357	1.000
D2	30min	pre	0min	.106	.320	1.000
			30min	-.060	.320	1.000
			60min	-.222	.320	1.000
			30min	.037	.357	1.000
	60min	pre	0min	.166	.320	1.000
			15min	.060	.320	1.000
			60min	-.162	.320	1.000
			60min	.199	.357	1.000
	15min	pre	0min	.328	.320	1.000
			15min	.222	.320	1.000
			30min	.162	.320	1.000
			0min	.176	.357	1.000
	15min	pre	15min	-.064	.357	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
0min	pre	30min		-.035	.357	1.000
			60min	-.137	.357	1.000
		15min		-.240	.320	1.000
		30min		-.211	.320	1.000
		60min		-.313	.320	1.000
	15min	pre		.064	.357	1.000
		0min		.240	.320	1.000
		30min		.029	.320	1.000
		60min		-.073	.320	1.000
		30min	pre	.035	.357	1.000
D4	pre	0min		.211	.320	1.000
			15min	-.029	.320	1.000
		15min	60min	-.102	.320	1.000
			pre	.137	.357	1.000
			0min	.313	.320	1.000
	0min	15min		.073	.320	1.000
			30min	.102	.320	1.000
		0min	pre	-.099	.479	1.000
		15min	30min	-.241	.337	1.000
			60min	-.405	.357	1.000
		15min		.211	.357	1.000
		0min	pre	.099	.479	1.000
		30min	15min	-.142	.438	1.000
			30min	-.307	.452	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D6	15min	60min		.310	.452	1.000
			pre	.241	.337	1.000
		0min		.142	.438	1.000
		30min		-.165	.299	1.000
	30min	60min		.452	.299	1.000
			pre	.405	.357	1.000
		0min		.307	.452	1.000
		15min		.165	.299	1.000
	60min	60min		.616	.320	.556
			pre	-.211	.357	1.000
		0min		-.310	.452	1.000
		15min		-.452	.299	1.000
	D6	pre	30min	-.616	.320	.556
			0min	-.106	.357	1.000
			15min	.052	.357	1.000
			30min	-.127	.357	1.000
	0min	0min	60min	-.170	.357	1.000
			pre	.106	.357	1.000
			15min	.158	.320	1.000
			30min	-.021	.320	1.000
	15min	15min	60min	-.064	.320	1.000
			pre	-.052	.357	1.000
			0min	-.158	.320	1.000
			30min	-.179	.320	1.000
	60min	60min	60min	-.221	.320	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
30min		pre		.127	.357	1.000
			0min	.021	.320	1.000
			15min	.179	.320	1.000
			60min	-.042	.319	1.000
60min		pre		.170	.357	1.000
			0min	.064	.320	1.000
			15min	.221	.320	1.000
			30min	.042	.319	1.000

Table A.6: The full table of post-hoc pairwise comparisons for Egr1 mRNA, discussed in §4.4.4. Bonferroni correction for multiple comparisons was used. P-values (“Sig.”) are reported with the standard error (“Std. Error”). Mean differences for which p-values less than .05 are starred for emphasis.

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
OB	D1	pre	0min	-1.379*	.448	.025
			15min	-1.545*	.448	.007
			30min	-1.411*	.448	.020
			60min	-.901	.448	.460
	0min	pre		1.379*	.448	.025
			15min	-.166	.400	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			30min	-.032	.400	1.000
			60min	.478	.400	1.000
		15min	pre	1.545*	.448	.007
			0min	.166	.400	1.000
			30min	.134	.400	1.000
			60min	.644	.400	1.000
		30min	pre	1.411*	.448	.020
			0min	.032	.400	1.000
			15min	-.134	.400	1.000
			60min	.510	.400	1.000
		60min	pre	.901	.448	.460
			0min	-.478	.400	1.000
			15min	-.644	.400	1.000
			30min	-.510	.400	1.000
D2	pre	0min		-.844	.448	.616
			15min	-.430	.448	1.000
			30min	-.306	.448	1.000
			60min	.122	.448	1.000
		0min	pre	.844	.448	.616
			15min	.414	.400	1.000
			30min	.537	.400	1.000
			60min	.966	.400	.172
		15min	pre	.430	.448	1.000
			0min	-.414	.400	1.000
			30min	.124	.400	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D4	0min	60min		.552	.400	1.000
			pre	.306	.448	1.000
		0min		-.537	.400	1.000
		15min		-.124	.400	1.000
		60min		.428	.400	1.000
	15min	60min	pre	-.122	.448	1.000
		0min		-.966	.400	.172
		15min		-.552	.400	1.000
		30min		-.428	.400	1.000
		0min	pre	-.932	.565	1.000
D4	30min	0min		-.806	.391	.411
			15min	-.169	.398	1.000
		60min		.179	.398	1.000
			pre	.932	.565	1.000
			15min	.126	.565	1.000
	60min	30min		.764	.566	1.000
			60min	1.111	.566	.518
		0min	pre	.806	.391	.411
			15min	-.126	.565	1.000
			30min	.638	.398	1.000
D4	15min	60min		.985	.398	.145
			pre	.169	.398	1.000
		0min		-.764	.566	1.000
			15min	-.638	.398	1.000
			60min	.348	.400	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D6	pre	60min	pre	-.179	.398	1.000
		0min		-1.111	.566	.518
		15min		-.985	.398	.145
		30min		-.348	.400	1.000
	0min	0min		-.892	.448	.483
		15min		-.724	.448	1.000
		30min		-.929	.446	.390
		60min		-.118	.446	1.000
	15min	pre		.892	.448	.483
		15min		.168	.400	1.000
		30min		-.037	.398	1.000
		60min		.774	.398	.539
	30min	pre		.724	.448	1.000
		0min		-.168	.400	1.000
		30min		-.204	.398	1.000
		60min		.606	.398	1.000
	60min	pre		.929	.446	.390
		0min		.037	.398	1.000
		15min		.204	.398	1.000
		60min		.811	.391	.400
	HPC	pre		.118	.446	1.000
		0min		-.774	.398	.539
		15min		-.606	.398	1.000
		30min		-.811	.391	.400
	D1	pre	0min	-.757	.448	.931

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			15min	-.499	.448	1.000
			30min	-.636	.448	1.000
			60min	-.624	.448	1.000
0min		pre		.757	.448	.931
			15min	.258	.400	1.000
			30min	.121	.400	1.000
			60min	.133	.400	1.000
15min		pre		.499	.448	1.000
			0min	-.258	.400	1.000
			30min	-.137	.400	1.000
			60min	-.125	.400	1.000
30min		pre		.636	.448	1.000
			0min	-.121	.400	1.000
			15min	.137	.400	1.000
			60min	.011	.400	1.000
60min		pre		.624	.448	1.000
			0min	-.133	.400	1.000
			15min	.125	.400	1.000
			30min	-.011	.400	1.000
D2	pre	0min		.131	.448	1.000
			15min	-.487	.448	1.000
			30min	-.120	.448	1.000
			60min	-.331	.448	1.000
0min		pre		-.131	.448	1.000
			15min	-.618	.400	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	15min	30min	-.251	.400	1.000
			60min	-.462	.400	1.000
	Day 2	15min	pre	.487	.448	1.000
			0min	.618	.400	1.000
	Day 3	15min	30min	.367	.400	1.000
			60min	.155	.400	1.000
	Day 4	30min	pre	.120	.448	1.000
			0min	.251	.400	1.000
	Day 5	15min	15min	-.367	.400	1.000
			60min	-.212	.400	1.000
D2	Day 1	60min	pre	.331	.448	1.000
			0min	.462	.400	1.000
	Day 2	15min	15min	-.155	.400	1.000
			30min	.212	.400	1.000
	Day 3	D4	0min	-1.466	.565	.104
			15min	-.881	.391	.258
	Day 4	0min	30min	-1.115	.398	.058
			60min	-.923	.398	.219
	Day 5	0min	pre	1.466	.565	.104
			15min	.585	.565	1.000
D3	Day 1	30min	30min	.352	.566	1.000
			60min	.544	.566	1.000
	Day 2	15min	pre	.881	.391	.258
			0min	-.585	.565	1.000
	Day 3	30min	30min	-.234	.398	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.	
D6	pre	60min		-.042	.398	1.000	
			pre	1.115	.398	.058	
		30min	0min	-.352	.566	1.000	
			15min	.234	.398	1.000	
			60min	.192	.400	1.000	
	0min	60min	pre	.923	.398	.219	
			0min	-.544	.566	1.000	
			15min	.042	.398	1.000	
			30min	-.192	.400	1.000	
		0min	0min	-.398	.448	1.000	
D6	15min		15min	-.798	.448	.770	
			30min	-.527	.446	1.000	
			60min	-.912	.446	.426	
		0min	pre	.398	.448	1.000	
			15min	-.400	.400	1.000	
	30min		30min	-.129	.398	1.000	
			60min	-.514	.398	1.000	
			pre	.798	.448	.770	
			0min	.400	.400	1.000	
D6	60min	15min	30min	.271	.398	1.000	
			60min	-.114	.398	1.000	
		0min	pre	.527	.446	1.000	
			0min	.129	.398	1.000	
			15min	-.271	.398	1.000	
	30min		60min	-.385	.391	1.000	

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
CTX	D1	60min	pre	.912	.446	.426
			0min	.514	.398	1.000
			15min	.114	.398	1.000
			30min	.385	.391	1.000
	15min	0min	pre	-1.265	.448	.054
		15min	-1.094	.448	.158	
		30min	-1.142	.448	.119	
		60min	-.985	.448	.295	
	30min	0min	pre	1.265	.448	.054
		15min	.170	.400	1.000	
		30min	.123	.400	1.000	
		60min	.280	.400	1.000	
	60min	15min	pre	1.094	.448	.158
		0min	-.170	.400	1.000	
		30min	-.047	.400	1.000	
		60min	.110	.400	1.000	
	D2	30min	pre	1.142	.448	.119
		0min	-.123	.400	1.000	
		15min	.047	.400	1.000	
		60min	.157	.400	1.000	
	pre	60min	pre	.985	.448	.295
		0min	-.280	.400	1.000	
		15min	-.110	.400	1.000	
		30min	-.157	.400	1.000	
	D2	pre	0min	-.886	.448	.498

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			15min	-1.172	.448	.098
			30min	-1.041	.448	.215
			60min	-1.178	.448	.095
	0min	pre		.886	.448	.498
			15min	-.286	.400	1.000
			30min	-.155	.400	1.000
			60min	-.292	.400	1.000
	15min	pre		1.172	.448	.098
			0min	.286	.400	1.000
			30min	.131	.400	1.000
			60min	-.006	.400	1.000
	30min	pre		1.041	.448	.215
			0min	.155	.400	1.000
			15min	-.131	.400	1.000
			60min	-.137	.400	1.000
	60min	pre		1.178	.448	.095
			0min	.292	.400	1.000
			15min	.006	.400	1.000
			30min	.137	.400	1.000
D4	pre	0min		-.944	.565	.967
			15min	-.871	.391	.275
			30min	-.500	.398	1.000
			60min	-.482	.398	1.000
	0min	pre		.944	.565	.967
			15min	.073	.565	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D6	15min	pre	30min	.444	.566	1.000
			60min	.463	.566	1.000
	30min	pre	15min	.871	.391	.275
			0min	-.073	.565	1.000
	60min	pre	30min	.371	.398	1.000
			60min	.389	.398	1.000
	0min	pre	30min	.500	.398	1.000
			0min	-.444	.566	1.000
	15min	pre	15min	-.371	.398	1.000
			60min	.018	.400	1.000
D6	60min	pre	60min	.482	.398	1.000
			0min	-.463	.566	1.000
	30min	pre	15min	-.389	.398	1.000
			30min	-.018	.400	1.000
	0min	pre	0min	-.916	.448	.426
			15min	-.902	.448	.459
	15min	pre	30min	-.937	.422	.282
			60min	-.801	.482	.988
	30min	pre	0min	.916	.448	.426
			15min	.014	.400	1.000
D6	60min	pre	30min	-.020	.372	1.000
			60min	.115	.439	1.000
	0min	pre	15min	.902	.448	.459
			0min	-.014	.400	1.000
	30min	pre	30min	-.034	.372	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			60min	.101	.439	1.000
	30min	pre		.937	.422	.282
			0min	.020	.372	1.000
			15min	.034	.372	1.000
			60min	.135	.404	1.000
	60min	pre		.801	.482	.988
			0min	-.115	.439	1.000
			15min	-.101	.439	1.000
			30min	-.135	.404	1.000
STR	D1	pre	0min	-.076	.483	1.000
			15min	-.570	.448	1.000
			30min	-.686	.448	1.000
			60min	-.714	.448	1.000
	0min	pre		.076	.483	1.000
			15min	-.494	.440	1.000
			30min	-.610	.440	1.000
			60min	-.638	.440	1.000
	15min	pre		.570	.448	1.000
			0min	.494	.440	1.000
			30min	-.116	.400	1.000
			60min	-.144	.400	1.000
	30min	pre		.686	.448	1.000
			0min	.610	.440	1.000
			15min	.116	.400	1.000
			60min	-.028	.400	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D2	pre	60min	pre	.714	.448	1.000
		0min		.638	.440	1.000
		15min		.144	.400	1.000
		30min		.028	.400	1.000
	15min	0min		-.395	.448	1.000
		15min		-.809	.448	.729
		30min		-.625	.448	1.000
		60min		-.623	.448	1.000
	30min	0min	pre	.395	.448	1.000
		15min		-.414	.400	1.000
		30min		-.230	.400	1.000
		60min		-.228	.400	1.000
	60min	15min	pre	.809	.448	.729
		0min		.414	.400	1.000
		30min		.184	.400	1.000
		60min		.186	.400	1.000
	0min	30min	pre	.625	.448	1.000
		0min		.230	.400	1.000
		15min		-.184	.400	1.000
		60min		.002	.400	1.000
	30min	60min	pre	.623	.448	1.000
		0min		.228	.400	1.000
		15min		-.186	.400	1.000
		30min		-.002	.400	1.000
D4	pre	0min		-1.128	.565	.478

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			15min	-.566	.391	1.000
			30min	-.355	.398	1.000
			60min	-.425	.398	1.000
	0min	pre		1.128	.565	.478
			15min	.562	.565	1.000
			30min	.773	.566	1.000
			60min	.703	.566	1.000
	15min	pre		.566	.391	1.000
			0min	-.562	.565	1.000
			30min	.211	.398	1.000
			60min	.141	.398	1.000
	30min	pre		.355	.398	1.000
			0min	-.773	.566	1.000
			15min	-.211	.398	1.000
			60min	-.070	.400	1.000
	60min	pre		.425	.398	1.000
			0min	-.703	.566	1.000
			15min	-.141	.398	1.000
			30min	.070	.400	1.000
D6	pre	0min		-.781	.547	1.000
			15min	-.685	.547	1.000
			30min	-.578	.546	1.000
			60min	-.701	.546	1.000
	0min	pre		.781	.547	1.000
			15min	.096	.400	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			30min	.203	.398	1.000
			60min	.080	.398	1.000
		15min	pre	.685	.547	1.000
			0min	-.096	.400	1.000
			30min	.107	.398	1.000
			60min	-.016	.398	1.000
		30min	pre	.578	.546	1.000
			0min	-.203	.398	1.000
			15min	-.107	.398	1.000
			60min	-.123	.391	1.000
		60min	pre	.701	.546	1.000
			0min	-.080	.398	1.000
			15min	.016	.398	1.000
			30min	.123	.391	1.000
CER	D1	pre	0min	-.199	.448	1.000
			15min	-.386	.448	1.000
			30min	-.273	.448	1.000
			60min	-.110	.448	1.000
		0min	pre	.199	.448	1.000
			15min	-.186	.400	1.000
			30min	-.074	.400	1.000
			60min	.089	.400	1.000
		15min	pre	.386	.448	1.000
			0min	.186	.400	1.000
			30min	.112	.400	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			60min	.276	.400	1.000
		30min	pre	.273	.448	1.000
			0min	.074	.400	1.000
			15min	-.112	.400	1.000
			60min	.163	.400	1.000
		60min	pre	.110	.448	1.000
			0min	-.089	.400	1.000
			15min	-.276	.400	1.000
			30min	-.163	.400	1.000
D2	pre	0min		-.028	.448	1.000
			15min	.126	.448	1.000
			30min	.156	.448	1.000
			60min	-.027	.448	1.000
	0min	pre		.028	.448	1.000
			15min	.154	.400	1.000
			30min	.184	.400	1.000
			60min	.001	.400	1.000
	15min	pre		-.126	.448	1.000
			0min	-.154	.400	1.000
			30min	.030	.400	1.000
			60min	-.153	.400	1.000
	30min	pre		-.156	.448	1.000
			0min	-.184	.400	1.000
			15min	-.030	.400	1.000
			60min	-.183	.400	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D4	pre	60min	pre	.027	.448	1.000
			0min	-.001	.400	1.000
			15min	.153	.400	1.000
			30min	.183	.400	1.000
	0min	0min	pre	-.637	.594	1.000
		15min	pre	-.109	.404	1.000
		30min	pre	-.009	.439	1.000
		60min	pre	-.111	.439	1.000
	15min	0min	pre	.637	.594	1.000
		15min	pre	.528	.546	1.000
		30min	pre	.628	.566	1.000
		60min	pre	.526	.566	1.000
	30min	15min	pre	.109	.404	1.000
		0min	pre	-.528	.546	1.000
		30min	pre	.100	.372	1.000
		60min	pre	-.002	.372	1.000
	60min	30min	pre	.009	.439	1.000
		0min	pre	-.628	.566	1.000
		15min	pre	-.100	.372	1.000
		60min	pre	-.102	.400	1.000
	D6	60min	pre	.111	.439	1.000
		0min	pre	-.526	.566	1.000
		15min	pre	.002	.372	1.000
		30min	pre	.102	.400	1.000
	D6	pre	0min	-.504	.448	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			15min	-.208	.448	1.000
			30min	-.186	.446	1.000
			60min	-.111	.446	1.000
0min		pre		.504	.448	1.000
			15min	.296	.400	1.000
			30min	.317	.398	1.000
			60min	.393	.398	1.000
15min		pre		.208	.448	1.000
			0min	-.296	.400	1.000
			30min	.021	.398	1.000
			60min	.097	.398	1.000
30min		pre		.186	.446	1.000
			0min	-.317	.398	1.000
			15min	-.021	.398	1.000
			60min	.076	.391	1.000
60min		pre		.111	.446	1.000
			0min	-.393	.398	1.000
			15min	-.097	.398	1.000
			30min	-.076	.391	1.000

Table A.7: The full table of post-hoc pairwise comparisons for Fos mRNA, discussed in §4.4.4. Bonferroni correction for multiple comparisons was used. P-values (“Sig.”) are reported with the standard error (“Std. Error”). Mean differences for which p-values less than .05 are starred for emphasis.

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
OB	D1	pre	0min	-2.503*	.363	.000
			15min	-2.515*	.363	.000
			30min	-2.113*	.363	.000
			60min	-1.441*	.363	.001
	0min	pre	2.503*	.363	.000	
			15min	-.012	.325	1.000
			30min	.390	.325	1.000
			60min	1.063*	.325	.015
	15min	pre	2.515*	.363	.000	
			0min	.012	.325	1.000
			30min	.402	.325	1.000
			60min	1.075*	.325	.014
	30min	pre	2.113*	.363	.000	
			0min	-.390	.325	1.000
			15min	-.402	.325	1.000
			60min	.673	.325	.413
	60min	pre	1.441*	.363	.001	

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.	
D2	pre	0min		-1.063*	.325	.015	
			15min	-1.075*	.325	.014	
			30min	-.673	.325	.413	
		0min		-1.287*	.363	.006	
	post	15min		-.771	.363	.367	
			30min	-.331	.363	1.000	
			60min	-.128	.363	1.000	
		0min	pre	1.287*	.363	.006	
	post	15min		.516	.325	1.000	
			30min	.956*	.325	.041	
			60min	1.159*	.325	.006	
		15min	pre	.771	.363	.367	
D4	pre	0min		-.516	.325	1.000	
			30min	.440	.325	1.000	
			60min	.643	.325	.510	
		30min	pre	.331	.363	1.000	
	post		0min	-.956*	.325	.041	
			15min	-.440	.325	1.000	
			60min	.203	.325	1.000	
	60min	pre	.128	.363	1.000		
		post		0min	-1.159*	.325	.006
				15min	-.643	.325	.510
				30min	-.203	.325	1.000
	0min	pre	-1.065	.456	.216		
	15min		-.890*	.306	.041		

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			30min	-.190	.320	1.000
			60min	.175	.320	1.000
		0min	pre	1.065	.456	.216
			15min	.175	.456	1.000
			30min	.875	.460	.600
			60min	1.240	.460	.083
		15min	pre	.890*	.306	.041
			0min	-.175	.456	1.000
			30min	.700	.320	.311
			60min	1.065*	.320	.012
		30min	pre	.190	.320	1.000
			0min	-.875	.460	.600
			15min	-.700	.320	.311
			60min	.365	.325	1.000
		60min	pre	-.175	.320	1.000
			0min	-1.240	.460	.083
			15min	-1.065*	.320	.012
			30min	-.365	.325	1.000
D6	pre	0min		-1.563*	.363	.000
			15min	-1.587*	.363	.000
			30min	-1.223*	.359	.010
			60min	-.625	.359	.850
		0min	pre	1.563*	.363	.000
			15min	-.024	.325	1.000
			30min	.340	.320	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
HPC	D1	15min	60min	.938*	.320	.042
			pre	1.587*	.363	.000
			0min	.024	.325	1.000
		30min	30min	.364	.320	1.000
			60min	.962*	.320	.034
			pre	1.223*	.359	.010
	D2	30min	0min	-.340	.320	1.000
			15min	-.364	.320	1.000
			60min	.598	.306	.523
		60min	pre	.625	.359	.850
			0min	-.938*	.320	.042
			15min	-.962*	.320	.034
			30min	-.598	.306	.523
HPC	D1	0min	pre	-1.998*	.363	.000
			15min	-1.874*	.363	.000
			30min	-1.762*	.363	.000
			60min	-1.351*	.363	.003
	15min	0min	pre	1.998*	.363	.000
			15min	.124	.325	1.000
			30min	.236	.325	1.000
			60min	.647	.325	.496
	30min	15min	pre	1.874*	.363	.000
			0min	-.124	.325	1.000
			30min	.112	.325	1.000
			60min	.523	.325	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.	
D1	Day 1	30min	pre	1.762*	.363	.000	
			0min	-.236	.325	1.000	
			15min	-.112	.325	1.000	
			60min	.411	.325	1.000	
	Day 2	60min	pre	1.351*	.363	.003	
			0min	-.647	.325	.496	
			15min	-.523	.325	1.000	
			30min	-.411	.325	1.000	
	Day 3	D2	pre	0min	-.740	.363	.445
				15min	-1.039	.363	.053
				30min	-.596	.363	1.000
				60min	-.721	.363	.504
	Day 4	0min	pre	.740	.363	.445	
				15min	-.299	.325	1.000
				30min	.145	.325	1.000
				60min	.020	.325	1.000
	Day 5	15min	pre	1.039	.363	.053	
				0min	.299	.325	1.000
				30min	.444	.325	1.000
				60min	.319	.325	1.000
	Day 6	30min	pre	.596	.363	1.000	
				0min	-.145	.325	1.000
				15min	-.444	.325	1.000
				60min	-.125	.325	1.000
	Day 7	60min	pre	.721	.363	.504	

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.	
D4	pre	0min		-.020	.325	1.000	
			15min	-.319	.325	1.000	
			30min	.125	.325	1.000	
		60min		-1.175	.456	.116	
	0min	15min		-.974*	.306	.018	
			30min	-1.061*	.320	.013	
			60min	-.837	.320	.103	
		15min		1.175	.456	.116	
	15min	30min		.201	.456	1.000	
			60min	.114	.460	1.000	
			0min	.337	.460	1.000	
		30min		.974*	.306	.018	
D6	pre	0min		-.201	.456	1.000	
			30min	-.087	.320	1.000	
			60min	.136	.320	1.000	
		30min		1.061*	.320	.013	
	60min		0min	-.114	.460	1.000	
			15min	.087	.320	1.000	
			60min	.223	.325	1.000	
	15min		.837	.320	.103		
	15min	30min		-.337	.460	1.000	
			0min	-.136	.320	1.000	
			15min	-.223	.325	1.000	
		30min		-1.393*	.363	.002	
	pre	0min		-1.551*	.363	.000	

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			30min	-1.395*	.359	.002
			60min	-1.200*	.359	.012
0min		pre		1.393*	.363	.002
			15min	-.158	.325	1.000
			30min	-.002	.320	1.000
			60min	.193	.320	1.000
15min		pre		1.551*	.363	.000
			0min	.158	.325	1.000
			30min	.156	.320	1.000
			60min	.351	.320	1.000
30min		pre		1.395*	.359	.002
			0min	.002	.320	1.000
			15min	-.156	.320	1.000
			60min	.195	.306	1.000
60min		pre		1.200*	.359	.012
			0min	-.193	.320	1.000
			15min	-.351	.320	1.000
			30min	-.195	.306	1.000
CTX	D1	pre	0min	-2.089*	.363	.000
			15min	-1.908*	.363	.000
			30min	-1.877*	.363	.000
			60min	-1.539*	.363	.001
0min		pre		2.089*	.363	.000
			15min	.181	.325	1.000
			30min	.212	.325	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			60min	.550	.325	.941
		15min	pre	1.908*	.363	.000
			0min	-.181	.325	1.000
			30min	.031	.325	1.000
			60min	.369	.325	1.000
		30min	pre	1.877*	.363	.000
			0min	-.212	.325	1.000
			15min	-.031	.325	1.000
			60min	.338	.325	1.000
		60min	pre	1.539*	.363	.001
			0min	-.550	.325	.941
			15min	-.369	.325	1.000
			30min	-.338	.325	1.000
D2	pre	0min		-1.235*	.363	.010
			15min	-1.453*	.363	.001
			30min	-1.237*	.363	.010
			60min	-1.163*	.363	.019
		0min	pre	1.235*	.363	.010
			15min	-.218	.325	1.000
			30min	-.001	.325	1.000
			60min	.072	.325	1.000
		15min	pre	1.453*	.363	.001
			0min	.218	.325	1.000
			30min	.217	.325	1.000
			60min	.290	.325	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D4	30min	30min	pre	1.237*	.363	.010
		0min		.001	.325	1.000
		15min		-.217	.325	1.000
		60min		.073	.325	1.000
	60min	60min	pre	1.163*	.363	.019
		0min		-.072	.325	1.000
		15min		-.290	.325	1.000
		30min		-.073	.325	1.000
	pre	0min		-1.052	.456	.233
		15min		-.869	.306	.051
		30min		-.639	.320	.486
		60min		-.506	.320	1.000
	0min	pre		1.052	.456	.233
		15min		.183	.456	1.000
		30min		.412	.460	1.000
		60min		.546	.460	1.000
	15min	pre		.869	.306	.051
		0min		-.183	.456	1.000
		30min		.229	.320	1.000
		60min		.363	.320	1.000
	30min	pre		.639	.320	.486
		0min		-.412	.460	1.000
		15min		-.229	.320	1.000
		60min		.134	.325	1.000
	60min	pre		.506	.320	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D6	pre	0min		-.546	.460	1.000
			15min	-.363	.320	1.000
			30min	-.134	.325	1.000
		0min		-1.811*	.363	.000
		15min		-1.647*	.363	.000
		30min		-1.681*	.341	.000
		60min		-1.155*	.385	.033
		0min	pre	1.811*	.363	.000
		15min		.164	.325	1.000
		30min		.130	.300	1.000
		60min		.656	.349	.623
		15min	pre	1.647*	.363	.000
STR	D1	0min		-.164	.325	1.000
			30min	-.034	.300	1.000
			60min	.492	.349	1.000
		30min	pre	1.681*	.341	.000
		0min		-.130	.300	1.000
		15min		.034	.300	1.000
		60min		.526	.308	.903
		60min	pre	1.155*	.385	.033
		0min		-.656	.349	.623
		15min		-.492	.349	1.000
		30min		-.526	.308	.903
		0min	pre	-1.002	.430	.213
		15min		-1.212	.430	.056

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
0min	D1	30min		-.775	.430	.738
			60min	-.680	.430	1.000
		pre		1.002	.430	.213
		15min		-.210	.325	1.000
		30min		.227	.325	1.000
	D2	60min		.322	.325	1.000
		15min	pre	1.212	.430	.056
		0min		.210	.325	1.000
		30min		.437	.325	1.000
		60min		.532	.325	1.000
30min	D1	30min	pre	.775	.430	.738
		0min		-.227	.325	1.000
		15min		-.437	.325	1.000
		60min		.095	.325	1.000
		60min	pre	.680	.430	1.000
	D2	0min		-.322	.325	1.000
		15min		-.532	.325	1.000
		30min		-.095	.325	1.000
		0min	pre	-.624	.363	.892
		15min		-.500	.363	1.000
60min	D2	30min		-.986	.363	.079
		60min		-.140	.363	1.000
		0min	pre	.624	.363	.892
		15min		.125	.325	1.000
		30min		-.362	.325	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	15min	60min	.484	.325	1.000
			pre	.500	.363	1.000
			0min	-.125	.325	1.000
			30min	-.487	.325	1.000
	Day 2	30min	60min	.359	.325	1.000
			pre	.986	.363	.079
			0min	.362	.325	1.000
			15min	.487	.325	1.000
	Day 3	60min	60min	.846	.325	.108
			pre	.140	.363	1.000
			0min	-.484	.325	1.000
			15min	-.359	.325	1.000
D4	Day 4	0min	30min	-.846	.325	.108
			pre	-1.126	.474	.194
			15min	-1.036*	.331	.021
			30min	-.718	.346	.397
	Day 5	15min	60min	-.303	.346	1.000
			pre	1.126	.474	.194
			15min	.089	.456	1.000
			30min	.407	.460	1.000
	Day 6	30min	60min	.823	.460	.768
			pre	1.036*	.331	.021
			0min	-.089	.456	1.000
			30min	.318	.320	1.000
	Day 7	60min	60min	.733	.320	.241

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D6	30min	30min	pre	.718	.346	.397
		0min		-.407	.460	1.000
		15min		-.318	.320	1.000
		60min		.415	.325	1.000
	60min	60min	pre	.303	.346	1.000
		0min		-.823	.460	.768
		15min		-.733	.320	.241
		30min		-.415	.325	1.000
	pre	0min		-1.105	.430	.113
		15min		-1.200	.430	.061
		30min		-1.006	.426	.197
		60min		-.566	.426	1.000
	0min	0min	pre	1.105	.430	.113
		15min		-.095	.325	1.000
		30min		.099	.320	1.000
		60min		.539	.320	.957
	15min	15min	pre	1.200	.430	.061
		0min		.095	.325	1.000
		30min		.193	.320	1.000
		60min		.633	.320	.507
	30min	30min	pre	1.006	.426	.197
		0min		-.099	.320	1.000
		15min		-.193	.320	1.000
		60min		.440	.306	1.000
	60min	60min	pre	.566	.426	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
CER	D1	pre	0min	-.539	.320	.957
			15min	-.633	.320	.507
			30min	-.440	.306	1.000
			60min	-2.897*	.363	.000
		pre	15min	-3.089*	.363	.000
			30min	-2.690*	.363	.000
			60min	-2.271*	.363	.000
			0min	2.897*	.363	.000
		pre	15min	-.192	.325	1.000
			30min	.207	.325	1.000
			60min	.626	.325	.573
			15min	3.089*	.363	.000
D2	D2	pre	0min	.192	.325	1.000
			30min	.399	.325	1.000
			60min	.818	.325	.136
			30min	2.690*	.363	.000
		pre	0min	-.207	.325	1.000
			15min	-.399	.325	1.000
			60min	.419	.325	1.000
			60min	2.271*	.363	.000
		pre	0min	-.626	.325	.573
			15min	-.818	.325	.136
			30min	-.419	.325	1.000
			0min	-1.656*	.363	.000
		pre	15min	-1.573*	.363	.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
			30min	-.891	.363	.162
			60min	-1.205*	.363	.013
		0min	pre	1.656*	.363	.000
			15min	.082	.325	1.000
			30min	.765	.325	.207
			60min	.451	.325	1.000
		15min	pre	1.573*	.363	.000
			0min	-.082	.325	1.000
			30min	.683	.325	.384
			60min	.368	.325	1.000
		30min	pre	.891	.363	.162
			0min	-.765	.325	.207
			15min	-.683	.325	.384
			60min	-.314	.325	1.000
		60min	pre	1.205*	.363	.013
			0min	-.451	.325	1.000
			15min	-.368	.325	1.000
			30min	.314	.325	1.000
D4	pre	0min		-1.727*	.477	.005
			15min	-.720	.309	.211
			30min	-1.152*	.349	.013
			60min	-.801	.349	.235
		0min	pre	1.727*	.477	.005
			15min	1.007	.442	.251
			30min	.574	.460	1.000

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
D1	Day 1	15min	60min	.926	.460	.470
			pre	.720	.309	.211
		Day 2	0min	-1.007	.442	.251
			30min	-.432	.300	1.000
			60min	-.081	.300	1.000
	Day 3	30min	pre	1.152*	.349	.013
			0min	-.574	.460	1.000
		Day 4	15min	.432	.300	1.000
			60min	.352	.325	1.000
			60min	.801	.349	.235
D6	Day 1	0min	0min	-.926	.460	.470
			15min	.081	.300	1.000
		Day 2	30min	-.352	.325	1.000
			0min	-1.745*	.363	.000
			15min	-1.516*	.363	.001
	Day 3	Day 4	30min	-1.323*	.359	.004
			60min	-.829	.359	.231
			0min	1.745*	.363	.000
		Day 5	15min	.229	.325	1.000
			30min	.422	.320	1.000
D12	Day 1	Day 6	60min	.916	.320	.051
			15min	1.516*	.363	.001
			0min	-.229	.325	1.000
		Day 7	30min	.193	.320	1.000
			60min	.687	.320	.342

Delay	Day	(A) Delay	(B) Delay	(A-B) Mean Difference	Std. Error	Sig.
30min		pre		1.323*	.359	.004
			0min	-.422	.320	1.000
			15min	-.193	.320	1.000
			60min	.494	.306	1.000
60min		pre		.829	.359	.231
			0min	-.916	.320	.051
			15min	-.687	.320	.342
			30min	-.494	.306	1.000

Table A.8: The full table of post-hoc pairwise comparisons for all genes on Day 1 of training discussed in §4.4.5. Bonferroni correction for multiple comparisons was used. P- values (“Sig.”) are reported with the standard error (“Std. Error”). Mean differences for which p-values less than .05 are starred for emphasis.

Region	Delay	(A)Gene	(B)Gene	(A-B)Mean Difference	Std Error	Sig
OB	pre	Arc	BDNF	1.429*	.371	.003
			Creb	.500	.371	1.000
			Egr1	.263	.371	1.000
			Erk1	-1.682*	.371	.000
			Erk2	-3.111*	.371	.000
			Fos	1.182*	.371	.034

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
BDNF		Bdnf	Arc	-1.429*	.371	.003
			Creb	-.929	.371	.271
			Egr1	-1.166*	.371	.039
			Erk1	-3.111*	.371	.000
			Erk2	-4.540*	.371	.000
			Fos	-.247	.371	1.000
Creb		Creb	Arc	-.500	.371	1.000
			Bdnf	.929	.371	.271
			Egr1	-.237	.371	1.000
			Erk1	-2.182*	.371	.000
			Erk2	-3.611*	.371	.000
			Fos	.682	.371	1.000
Egr1		Egr1	Arc	-.263	.371	1.000
			Bdnf	1.166*	.371	.039
			Creb	.237	.371	1.000
			Erk1	-1.945*	.371	.000
			Erk2	-3.374*	.371	.000
			Fos	.919	.371	.292
Erk1		Erk1	Arc	1.682*	.371	.000
			Bdnf	3.111*	.371	.000
			Creb	2.182*	.371	.000
			Egr1	1.945*	.371	.000
			Erk2	-1.429*	.371	.003
			Fos	2.865*	.371	.000
Erk2		Erk2	Arc	3.111*	.371	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1hr	Fos	BDNF	4.540*	.371	.000
			Creb	3.611*	.371	.000
			Egr1	3.374*	.371	.000
			Erk1	1.429*	.371	.003
			Fos	4.294*	.371	.000
Amygdala	2hr	Fos	Arc	-1.182*	.371	.034
			BDNF	.247	.371	1.000
			Creb	-.682	.371	1.000
			Egr1	-.919	.371	.292
			Erk1	-2.865*	.371	.000
			Erk2	-4.294*	.371	.000
Amygdala	4hr	Arc	BDNF	2.921*	.303	.000
			Creb	2.088*	.303	.000
			Egr1	.673	.303	.574
			Erk1	.240	.340	1.000
			Erk2	-1.165*	.340	.015
			Fos	.468	.303	1.000
Amygdala	8hr	Arc	BDNF	-2.921*	.303	.000
			Creb	-.833	.303	.134
			Egr1	-2.248*	.303	.000
			Erk1	-2.681*	.340	.000
			Erk2	-4.086*	.340	.000
			Fos	-2.453*	.303	.000
Amygdala	24hr	Creb	Arc	-2.088*	.303	.000
			BDNF	.833	.303	.134

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1 hr	Egr1		-1.415*	.303	.000
		Erk1		-1.848*	.340	.000
		Erk2		-3.253*	.340	.000
		Fos		-1.620*	.303	.000
Prefrontal cortex	1 hr	Egr1	Arc	-.673	.303	.574
			BDNF	2.248*	.303	.000
			Creb	1.415*	.303	.000
			Erk1	-.433	.340	1.000
			Erk2	-1.838*	.340	.000
			Fos	-.205	.303	1.000
Prefrontal cortex	24 hr	Erk1	Arc	-.240	.340	1.000
			BDNF	2.681*	.340	.000
			Creb	1.848*	.340	.000
			Egr1	.433	.340	1.000
			Erk2	-1.405*	.371	.004
			Fos	.228	.340	1.000
Prefrontal cortex	48 hr	Erk2	Arc	1.165*	.340	.015
			BDNF	4.086*	.340	.000
			Creb	3.253*	.340	.000
			Egr1	1.838*	.340	.000
			Erk1	1.405*	.371	.004
			Fos	1.633*	.340	.000
Amygdala	48 hr	Fos	Arc	-.468	.303	1.000
			BDNF	2.453*	.303	.000
			Creb	1.620*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
			Egr1	.205	.303	1.000
			Erk1	-.228	.340	1.000
			Erk2	-1.633*	.340	.000
15min	Arc		BDNF	2.794*	.303	.000
			Creb	2.073*	.303	.000
			Egr1	.408	.303	1.000
			Erk1	-.005	.303	1.000
			Erk2	-1.380*	.303	.000
			Fos	.358	.303	1.000
	BDNF		Arc	-2.794*	.303	.000
			Creb	-.721	.303	.379
			Egr1	-2.385*	.303	.000
			Erk1	-2.798*	.303	.000
			Erk2	-4.173*	.303	.000
			Fos	-2.436*	.303	.000
	Creb		Arc	-2.073*	.303	.000
			BDNF	.721	.303	.379
			Egr1	-1.664*	.303	.000
			Erk1	-2.077*	.303	.000
			Erk2	-3.452*	.303	.000
			Fos	-1.715*	.303	.000
	Egr1		Arc	-.408	.303	1.000
			BDNF	2.385*	.303	.000
			Creb	1.664*	.303	.000
			Erk1	-.413	.303	1.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
			Erk2	-1.788*	.303	.000
			Fos	-.051	.303	1.000
		Erk1	Arc	.005	.303	1.000
			BDNF	2.798*	.303	.000
			Creb	2.077*	.303	.000
			Egr1	.413	.303	1.000
			Erk2	-1.375*	.303	.000
			Fos	.362	.303	1.000
		Erk2	Arc	1.380*	.303	.000
			BDNF	4.173*	.303	.000
			Creb	3.452*	.303	.000
			Egr1	1.788*	.303	.000
			Erk1	1.375*	.303	.000
			Fos	1.737*	.303	.000
		Fos	Arc	-.358	.303	1.000
			BDNF	2.436*	.303	.000
			Creb	1.715*	.303	.000
			Egr1	.051	.303	1.000
			Erk1	-.362	.303	1.000
			Erk2	-1.737*	.303	.000
	30min	Arc	BDNF	2.482*	.303	.000
			Creb	1.636*	.303	.000
			Egr1	.122	.303	1.000
			Erk1	-.316	.303	1.000
			Erk2	-1.743*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
		Fos		.339	.303	1.000
BDNF		Arc		-2.482*	.303	.000
		Creb		-.846	.303	.118
		Egr1		-2.360*	.303	.000
		Erk1		-2.798*	.303	.000
		Erk2		-4.226*	.303	.000
		Fos		-2.143*	.303	.000
Creb		Arc		-1.636*	.303	.000
		BDNF		.846	.303	.118
		Egr1		-1.514*	.303	.000
		Erk1		-1.952*	.303	.000
		Erk2		-3.379*	.303	.000
		Fos		-1.297*	.303	.001
Egr1		Arc		-.122	.303	1.000
		BDNF		2.360*	.303	.000
		Creb		1.514*	.303	.000
		Erk1		-.438	.303	1.000
		Erk2		-1.866*	.303	.000
		Fos		.217	.303	1.000
Erk1		Arc		.316	.303	1.000
		BDNF		2.798*	.303	.000
		Creb		1.952*	.303	.000
		Egr1		.438	.303	1.000
		Erk2		-1.427*	.303	.000
		Fos		.655	.303	.662

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1hr	Erk2	Arc	1.743*	.303	.000
			BDNF	4.226*	.303	.000
			Creb	3.379*	.303	.000
			Egr1	1.866*	.303	.000
			Erk1	1.427*	.303	.000
			Fos	2.082*	.303	.000
Amygdala	2hr	Fos	Arc	-.339	.303	1.000
			BDNF	2.143*	.303	.000
			Creb	1.297*	.303	.001
			Egr1	-.217	.303	1.000
			Erk1	-.655	.303	.662
			Erk2	-2.082*	.303	.000
Amygdala	60min	Arc	BDNF	2.069*	.303	.000
			Creb	1.338*	.303	.000
			Egr1	.368	.303	1.000
			Erk1	-.515	.303	1.000
			Erk2	-1.957*	.303	.000
			Fos	.748	.303	.299
Amygdala	1hr	BDNF	Arc	-2.069*	.303	.000
			Creb	-.731	.303	.347
			Egr1	-1.701*	.303	.000
			Erk1	-2.584*	.303	.000
			Erk2	-4.026*	.303	.000
			Fos	-1.321*	.303	.000
Amygdala	2hr	Creb	Arc	-1.338*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1 hr		BDNF	.731	.303	.347
			Egr1	-.970*	.303	.032
			Erk1	-1.853*	.303	.000
			Erk2	-3.295*	.303	.000
			Fos	-.590	.303	1.000
		Egr1	Arc	-.368	.303	1.000
Prefrontal cortex	1 hr		BDNF	1.701*	.303	.000
			Creb	.970*	.303	.032
			Erk1	-.883	.303	.081
			Erk2	-2.325*	.303	.000
			Fos	.380	.303	1.000
		Erk1	Arc	.515	.303	1.000
Amygdala	24 hr		BDNF	2.584*	.303	.000
			Creb	1.853*	.303	.000
			Egr1	.883	.303	.081
			Erk2	-1.442*	.303	.000
			Fos	1.263*	.303	.001
		Erk2	Arc	1.957*	.303	.000
Prefrontal cortex	24 hr		BDNF	4.026*	.303	.000
			Creb	3.295*	.303	.000
			Egr1	2.325*	.303	.000
			Erk1	1.442*	.303	.000
			Fos	2.705*	.303	.000
		Fos	Arc	-.748	.303	.299
			BDNF	1.321*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.	
HPC	pre	Arc	Creb	.590	.303	1.000	
			Egr1	-.380	.303	1.000	
			Erk1	-1.263*	.303	.001	
			Erk2	-2.705*	.303	.000	
			BDNF	.155	.371	1.000	
			Creb	1.191*	.371	.032	
			Egr1	.737	.371	1.000	
			Erk1	-.933	.371	.263	
			Erk2	-2.560*	.371	.000	
			Fos	2.266*	.371	.000	
			BDNF	Arc	-.155	.371	1.000
			Creb	1.036	.371	.119	
			Egr1	.582	.371	1.000	
			Erk1	-1.088	.371	.077	
			Erk2	-2.716*	.371	.000	
			Fos	2.111*	.371	.000	
			Creb	Arc	-1.191*	.371	.032
			BDNF	-1.036	.371	.119	
			Egr1	-.453	.371	1.000	
			Erk1	-2.124*	.371	.000	
			Erk2	-3.751*	.371	.000	
			Fos	1.075	.371	.086	
			Egr1	Arc	-.737	.371	1.000
			BDNF	-.582	.371	1.000	
			Creb	.453	.371	1.000	

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
			Erk1	-1.670*	.371	.000
			Erk2	-3.298*	.371	.000
			Fos	1.529*	.371	.001
		Erk1	Arc	.933	.371	.263
		BDNF	1.088	.371	.077	
		Creb	2.124*	.371	.000	
		Egr1	1.670*	.371	.000	
		Erk2	-1.627*	.371	.000	
		Fos	3.199*	.371	.000	
		Erk2	Arc	2.560*	.371	.000
		BDNF	2.716*	.371	.000	
		Creb	3.751*	.371	.000	
		Egr1	3.298*	.371	.000	
		Erk1	1.627*	.371	.000	
		Fos	4.826*	.371	.000	
		Fos	Arc	-2.266*	.371	.000
		BDNF	-2.111*	.371	.000	
		Creb	-1.075	.371	.086	
		Egr1	-1.529*	.371	.001	
		Erk1	-3.199*	.371	.000	
		Erk2	-4.826*	.371	.000	
	0min	Arc	BDNF	2.436*	.303	.000
			Creb	2.281*	.303	.000
			Egr1	1.038*	.303	.015
			Erk1	-.009	.303	1.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
			Erk2	-1.567*	.303	.000
			Fos	1.326*	.303	.000
		BDNF	Arc	-2.436*	.303	.000
			Creb	-.155	.303	1.000
			Egr1	-1.397*	.303	.000
			Erk1	-2.445*	.303	.000
			Erk2	-4.003*	.303	.000
			Fos	-1.110*	.303	.006
		Creb	Arc	-2.281*	.303	.000
			BDNF	.155	.303	1.000
			Egr1	-1.242*	.303	.001
			Erk1	-2.290*	.303	.000
			Erk2	-3.848*	.303	.000
			Fos	-.955*	.303	.038
		Egr1	Arc	-1.038*	.303	.015
			BDNF	1.397*	.303	.000
			Creb	1.242*	.303	.001
			Erk1	-1.048*	.303	.013
			Erk2	-2.606*	.303	.000
			Fos	.287	.303	1.000
		Erk1	Arc	.009	.303	1.000
			BDNF	2.445*	.303	.000
			Creb	2.290*	.303	.000
			Egr1	1.048*	.303	.013
			Erk2	-1.558*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
		Fos		1.335*	.303	.000
		Erk2	Arc	1.567*	.303	.000
			BDNF	4.003*	.303	.000
			Creb	3.848*	.303	.000
			Egr1	2.606*	.303	.000
			Erk1	1.558*	.303	.000
			Fos	2.893*	.303	.000
		Fos	Arc	-1.326*	.303	.000
			BDNF	1.110*	.303	.006
			Creb	.955*	.303	.038
			Egr1	-.287	.303	1.000
			Erk1	-1.335*	.303	.000
			Erk2	-2.893*	.303	.000
15min		Arc	BDNF	1.590*	.303	.000
			Creb	2.373*	.303	.000
			Egr1	1.579*	.303	.000
			Erk1	.458	.303	1.000
			Erk2	-1.173*	.303	.003
			Fos	1.733*	.303	.000
		BDNF	Arc	-1.590*	.303	.000
			Creb	.783	.303	.217
			Egr1	-.011	.303	1.000
			Erk1	-1.132*	.303	.005
			Erk2	-2.763*	.303	.000
			Fos	.143	.303	1.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1 hr	Creb	Arc	-2.373*	.303	.000
			BDNF	-.783	.303	.217
			Egr1	-.793	.303	.196
			Erk1	-1.915*	.303	.000
			Erk2	-3.546*	.303	.000
			Fos	-.640	.303	.747
Amygdala	24 hr	Egr1	Arc	-1.579*	.303	.000
			BDNF	.011	.303	1.000
			Creb	.793	.303	.196
			Erk1	-1.121*	.303	.006
			Erk2	-2.753*	.303	.000
			Fos	.153	.303	1.000
Amygdala	48 hr	Erk1	Arc	-.458	.303	1.000
			BDNF	1.132*	.303	.005
			Creb	1.915*	.303	.000
			Egr1	1.121*	.303	.006
			Erk2	-1.632*	.303	.000
			Fos	1.274*	.303	.001
Amygdala	72 hr	Erk2	Arc	1.173*	.303	.003
			BDNF	2.763*	.303	.000
			Creb	3.546*	.303	.000
			Egr1	2.753*	.303	.000
			Erk1	1.632*	.303	.000
			Fos	2.906*	.303	.000
Amygdala	144 hr	Fos	Arc	-1.733*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.	
30min	Arc		BDNF	-.143	.303	1.000	
			Creb	.640	.303	.747	
			Egr1	-.153	.303	1.000	
			Erk1	-1.274*	.303	.001	
			Erk2	-2.906*	.303	.000	
			BDNF	1.376*	.303	.000	
			Creb	2.268*	.303	.000	
			Egr1	1.341*	.303	.000	
			Erk1	.316	.303	1.000	
			Erk2	-1.348*	.303	.000	
			Fos	1.743*	.303	.000	
			BDNF	Arc	-1.376*	.303	.000
			Creb	.892	.303	.075	
			Egr1	-.035	.303	1.000	
			Erk1	-1.060*	.303	.012	
			Erk2	-2.724*	.303	.000	
			Fos	.367	.303	1.000	
			Creb	Arc	-2.268*	.303	.000
			BDNF		-.892	.303	.075
			Egr1		-.927	.303	.051
			Erk1		-1.951*	.303	.000
			Erk2		-3.615*	.303	.000
			Fos		-.525	.303	1.000
			Egr1	Arc	-1.341*	.303	.000
			BDNF		.035	.303	1.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1hr	Erk1	Creb	.927	.303	.051
			Erk1	-1.024*	.303	.017
			Erk2	-2.688*	.303	.000
			Fos	.402	.303	1.000
	2hr	Erk2	Arc	-.316	.303	1.000
			BDNF	1.060*	.303	.012
			Creb	1.951*	.303	.000
			Egr1	1.024*	.303	.017
			Erk2	-1.664*	.303	.000
			Fos	1.426*	.303	.000
Prefrontal Cortex	1hr	Erk2	Arc	1.348*	.303	.000
			BDNF	2.724*	.303	.000
			Creb	3.615*	.303	.000
			Egr1	2.688*	.303	.000
			Erk1	1.664*	.303	.000
			Fos	3.090*	.303	.000
Prefrontal Cortex	2hr	Fos	Arc	-1.743*	.303	.000
			BDNF	-.367	.303	1.000
			Creb	.525	.303	1.000
			Egr1	-.402	.303	1.000
			Erk1	-1.426*	.303	.000
			Erk2	-3.090*	.303	.000
Amygdala	60min	Arc	BDNF	1.156*	.303	.004
			Creb	1.960*	.303	.000
			Egr1	1.142*	.303	.004

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1 hr		Erk1	.105	.303	1.000
			Erk2	-1.550*	.303	.000
			Fos	1.943*	.303	.000
Prefrontal cortex	1 hr	BDNF	Arc	-1.156*	.303	.004
			Creb	.803	.303	.179
			Egr1	-.014	.303	1.000
			Erk1	-1.052*	.303	.013
			Erk2	-2.706*	.303	.000
			Fos	.787	.303	.209
Amygdala	1 hr	Creb	Arc	-1.960*	.303	.000
			BDNF	-.803	.303	.179
			Egr1	-.818	.303	.156
			Erk1	-1.855*	.303	.000
			Erk2	-3.509*	.303	.000
			Fos	-.016	.303	1.000
Prefrontal cortex	1 hr	Egr1	Arc	-1.142*	.303	.004
			BDNF	.014	.303	1.000
			Creb	.818	.303	.156
			Erk1	-1.037*	.303	.015
			Erk2	-2.692*	.303	.000
			Fos	.801	.303	.182
Amygdala	1 hr	Erk1	Arc	-.105	.303	1.000
			BDNF	1.052*	.303	.013
			Creb	1.855*	.303	.000
			Egr1	1.037*	.303	.015

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.		
			Erk2	-1.654*	.303	.000		
			Fos	1.839*	.303	.000		
		Erk2	Arc	1.550*	.303	.000		
		BDNF	2.706*	.303	.000			
		Creb	3.509*	.303	.000			
		Egr1	2.692*	.303	.000			
		Erk1	1.654*	.303	.000			
		Fos	3.493*	.303	.000			
		Fos	Arc	-1.943*	.303	.000		
		BDNF	-.787	.303	.209			
		Creb	.016	.303	1.000			
		Egr1	-.801	.303	.182			
		Erk1	-1.839*	.303	.000			
		Erk2	-3.493*	.303	.000			
		CTX	pre	Arc	BDNF	.763	.371	.859
		Creb	.322	.371	1.000			
		Egr1	.135	.371	1.000			
		Erk1	-.100	.371	1.000			
		Erk2	-2.464*	.371	.000			
		Fos	2.149*	.371	.000			
			BDNF	Arc	-.763	.371	.859	
			Creb	-.441	.371	1.000		
			Egr1	-.628	.371	1.000		
			Erk1	-.863	.371	.438		
			Erk2	-3.227*	.371	.000		

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
		Fos		1.387*	.371	.005
Cerb		Arc		-.322	.371	1.000
		BDNF		.441	.371	1.000
		Egr1		-.187	.371	1.000
		Erk1		-.422	.371	1.000
		Erk2		-2.786*	.371	.000
		Fos		1.827*	.371	.000
Egr1		Arc		-.135	.371	1.000
		BDNF		.628	.371	1.000
		Cerb		.187	.371	1.000
		Erk1		-.235	.371	1.000
		Erk2		-2.599*	.371	.000
		Fos		2.014*	.371	.000
Erk1		Arc		.100	.371	1.000
		BDNF		.863	.371	.438
		Cerb		.422	.371	1.000
		Egr1		.235	.371	1.000
		Erk2		-2.364*	.371	.000
		Fos		2.249*	.371	.000
Erk2		Arc		2.464*	.371	.000
		BDNF		3.227*	.371	.000
		Cerb		2.786*	.371	.000
		Egr1		2.599*	.371	.000
		Erk1		2.364*	.371	.000
		Fos		4.613*	.371	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
0min	Arc	Fos	Arc	-2.149*	.371	.000
			BDNF	-1.387*	.371	.005
			Creb	-1.827*	.371	.000
			Egr1	-2.014*	.371	.000
			Erk1	-2.249*	.371	.000
			Erk2	-4.613*	.371	.000
0min	BDNF		BDNF	2.288*	.303	.000
			Creb	2.202*	.303	.000
			Egr1	.516	.303	1.000
			Erk1	1.525*	.303	.000
			Erk2	-.637	.303	.766
			Fos	1.706*	.303	.000
0min	Creb		Arc	-2.288*	.303	.000
			BDNF	-.087	.303	1.000
			Egr1	-1.773*	.303	.000
			Erk1	-.764	.303	.259
			Erk2	-2.926*	.303	.000
			Fos	-.582	.303	1.000
0min	Egr1		Arc	-2.202*	.303	.000
			BDNF	.087	.303	1.000
			Creb	-1.686*	.303	.000
			Erk1	-.677	.303	.553
			Erk2	-2.839*	.303	.000
			Fos	-.496	.303	1.000
0min	Erk1		Arc	-.516	.303	1.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.	
Amygdala	15min	Arc	BDNF	1.773*	.303	.000	
			Creb	1.686*	.303	.000	
			Erk1	1.009*	.303	.021	
			Erk2	-1.153*	.303	.004	
			Fos	1.190*	.303	.002	
		Erk1	Arc	-1.525*	.303	.000	
Prefrontal cortex	15min	Arc	BDNF	.764	.303	.259	
			Creb	.677	.303	.553	
			Egr1	-1.009*	.303	.021	
			Erk2	-2.162*	.303	.000	
			Fos	.181	.303	1.000	
		Erk2	Arc	.637	.303	.766	
Hippocampus	15min	Arc	BDNF	2.926*	.303	.000	
			Creb	2.839*	.303	.000	
			Egr1	1.153*	.303	.004	
			Erk1	2.162*	.303	.000	
			Fos	2.343*	.303	.000	
		Fos	Arc	-1.706*	.303	.000	
Striatum	15min	Arc	BDNF	.582	.303	1.000	
			Creb	.496	.303	1.000	
			Egr1	-1.190*	.303	.002	
			Erk1	-.181	.303	1.000	
			Erk2	-2.343*	.303	.000	
		15min	Arc	BDNF	2.773*	.303	.000
			Creb	2.885*	.303	.000	

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1 hr	Egr1		1.420*	.303	.000
		Erk1		2.284*	.303	.000
		Erk2		.012	.303	1.000
		Fos		2.621*	.303	.000
Prefrontal cortex	1 hr	BDNF	Arc	-2.773*	.303	.000
			Creb	.112	.303	1.000
		Egr1		-1.353*	.303	.000
		Erk1		-.489	.303	1.000
		Erk2		-2.761*	.303	.000
		Fos		-.152	.303	1.000
Prefrontal cortex	4 hr	Creb	Arc	-2.885*	.303	.000
			BDNF	-.112	.303	1.000
		Egr1		-1.464*	.303	.000
		Erk1		-.601	.303	1.000
		Erk2		-2.872*	.303	.000
		Fos		-.264	.303	1.000
Prefrontal cortex	24 hr	Egr1	Arc	-1.420*	.303	.000
			BDNF	1.353*	.303	.000
		Creb		1.464*	.303	.000
		Erk1		.863	.303	.099
		Erk2		-1.408*	.303	.000
		Fos		1.200*	.303	.002
Amygdala	24 hr	Erk1	Arc	-2.284*	.303	.000
			BDNF	.489	.303	1.000
		Creb		.601	.303	1.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.	
Amygdala	1hr		Egr1	-.863	.303	.099	
			Erk2	-2.271*	.303	.000	
			Fos	.337	.303	1.000	
Amygdala	2hr	Erk2	Arc	-.012	.303	1.000	
			BDNF	2.761*	.303	.000	
			Creb	2.872*	.303	.000	
			Egr1	1.408*	.303	.000	
			Erk1	2.271*	.303	.000	
			Fos	2.609*	.303	.000	
Amygdala	4hr	Fos	Arc	-2.621*	.303	.000	
			BDNF	.152	.303	1.000	
			Creb	.264	.303	1.000	
			Egr1	-1.200*	.303	.002	
			Erk1	-.337	.303	1.000	
			Erk2	-2.609*	.303	.000	
Amygdala	24hr	30min	Arc	BDNF	2.677*	.303	.000
				Creb	2.675*	.303	.000
				Egr1	1.302*	.303	.001
				Erk1	2.007*	.303	.000
				Erk2	-.116	.303	1.000
				Fos	2.581*	.303	.000
Amygdala	1hr		BDNF	Arc	-2.677*	.303	.000
				Creb	-.001	.303	1.000
				Egr1	-1.375*	.303	.000
				Erk1	-.670	.303	.586

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
			Erk2	-2.793*	.303	.000
			Fos	-.096	.303	1.000
		Crb	Arc	-2.675*	.303	.000
			BDNF	.001	.303	1.000
			Egr1	-1.374*	.303	.000
			Erk1	-.669	.303	.592
			Erk2	-2.791*	.303	.000
			Fos	-.095	.303	1.000
		Egr1	Arc	-1.302*	.303	.001
			BDNF	1.375*	.303	.000
			Crb	1.374*	.303	.000
			Erk1	.705	.303	.435
			Erk2	-1.418*	.303	.000
			Fos	1.279*	.303	.001
		Erk1	Arc	-2.007*	.303	.000
			BDNF	.670	.303	.586
			Crb	.669	.303	.592
			Egr1	-.705	.303	.435
			Erk2	-2.123*	.303	.000
			Fos	.574	.303	1.000
		Erk2	Arc	.116	.303	1.000
			BDNF	2.793*	.303	.000
			Crb	2.791*	.303	.000
			Egr1	1.418*	.303	.000
			Erk1	2.123*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	10min	Fos	Fos	2.697*	.303	.000
			Arc	-2.581*	.303	.000
			BDNF	.096	.303	1.000
			Creb	.095	.303	1.000
			Egr1	-1.279*	.303	.001
			Erk1	-.574	.303	1.000
			Erk2	-2.697*	.303	.000
		Arc	BDNF	2.383*	.303	.000
			Creb	2.468*	.303	.000
			Egr1	1.240*	.303	.001
			Erk1	1.861*	.303	.000
60min	60min	Erk2	Fos	-.306	.303	1.000
			BDNF	2.700*	.303	.000
			Arc	-2.383*	.303	.000
			Creb	.085	.303	1.000
			Egr1	-1.142*	.303	.004
			Erk1	-.522	.303	1.000
		Fos	Erk2	-2.688*	.303	.000
			Creb	-2.468*	.303	.000
			BDNF	-.085	.303	1.000
			Egr1	-1.227*	.303	.001
Hippocampus	10min	Creb	Erk1	-.607	.303	.973
			Erk2	-2.773*	.303	.000
		Fos		.233	.303	1.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
STR	pre	Egr1	Arc	-1.240*	.303	.001
			BDNF	1.142*	.303	.004
			Creb	1.227*	.303	.001
			Erk1	.621	.303	.871
			Erk2	-1.546*	.303	.000
	post		Fos	1.460*	.303	.000
		Erk1	Arc	-1.861*	.303	.000
			BDNF	.522	.303	1.000
			Creb	.607	.303	.973
			Egr1	-.621	.303	.871
Hippocampus	pre		Erk2	-2.167*	.303	.000
			Fos	.839	.303	.126
		Erk2	Arc	.306	.303	1.000
			BDNF	2.688*	.303	.000
			Creb	2.773*	.303	.000
	post		Egr1	1.546*	.303	.000
			Erk1	2.167*	.303	.000
			Fos	3.006*	.303	.000
		Fos	Arc	-2.700*	.303	.000
			BDNF	-.318	.303	1.000
Amygdala	pre		Creb	-.233	.303	1.000
			Egr1	-1.460*	.303	.000
			Erk1	-.839	.303	.126
	post		Erk2	-3.006*	.303	.000
		STR	pre	Arc	BDNF	.958
					.457	.771

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
BDNF	0	Creb		-.779	.371	.775
		Egr1		-.097	.371	1.000
		Erk1		-.625	.371	1.000
		Erk2		-3.125*	.371	.000
		Fos		.626	.457	1.000
	1	Arc		-.958	.457	.771
		Creb		-1.737*	.457	.004
		Egr1		-1.055	.457	.452
		Erk1		-1.583*	.457	.013
		Erk2		-4.083*	.457	.000
Creb	0	Fos		-.332	.525	1.000
		Arc		.779	.371	.775
		BDNF		1.737*	.457	.004
		Egr1		.682	.371	1.000
		Erk1		.154	.371	1.000
	1	Erk2		-2.346*	.371	.000
		Fos		1.405*	.457	.048
		Egr1		.097	.371	1.000
		BDNF		1.055	.457	.452
		Creb		-.682	.371	1.000
Egr1	0	Erk1		-.528	.371	1.000
		Erk2		-3.028*	.371	.000
		Fos		.723	.457	1.000
		Arc		.625	.371	1.000
		BDNF		1.583*	.457	.013

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1hr	Erk2	Creb	-.154	.371	1.000
			Egr1	.528	.371	1.000
			Erk2	-2.500*	.371	.000
			Fos	1.251	.457	.137
		Arc	BDNF	3.125*	.371	.000
	0min	Fos	Creb	4.083*	.457	.000
			Egr1	2.346*	.371	.000
			Erk1	3.028*	.371	.000
			Fos	2.500*	.371	.000
		Arc	BDNF	3.751*	.457	.000
Hippocampus	1hr	Fos	Creb	-.626	.457	1.000
			Egr1	.332	.525	1.000
			Erk1	-1.405*	.457	.048
			Erk2	-.723	.457	1.000
		Arc	BDNF	-1.251	.457	.137
	0min	Arc	Creb	-3.751*	.457	.000
			Egr1	2.768*	.303	.000
			Erk1	2.186*	.303	.000
			Erk2	1.465*	.340	.000
		BDNF	Fos	.567	.303	1.000
	10min	Arc	Erk2	-1.466*	.303	.000
			Fos	1.232*	.303	.001
		BDNF	Creb	-2.768*	.303	.000
		Fos	Egr1	-.581	.303	1.000
			Erk1	-1.303*	.340	.003

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
		Erk1		-2.201*	.303	.000
		Erk2		-4.234*	.303	.000
		Fos		-1.536*	.303	.000
		Creb	Arc	-2.186*	.303	.000
			BDNF	.581	.303	1.000
		Egr1		-.722	.340	.724
		Erk1		-1.619*	.303	.000
		Erk2		-3.653*	.303	.000
		Fos		-.954*	.303	.038
		Egr1	Arc	-1.465*	.340	.000
			BDNF	1.303*	.340	.003
		Creb		.722	.340	.724
		Erk1		-.897	.340	.183
		Erk2		-2.931*	.340	.000
		Fos		-.233	.340	1.000
		Erk1	Arc	-.567	.303	1.000
			BDNF	2.201*	.303	.000
		Creb		1.619*	.303	.000
		Egr1		.897	.340	.183
		Erk2		-2.033*	.303	.000
		Fos		.665	.303	.611
		Erk2	Arc	1.466*	.303	.000
			BDNF	4.234*	.303	.000
		Creb		3.653*	.303	.000
		Egr1		2.931*	.340	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
15min	Arc	Erk1	Erk1	2.033*	.303	.000
			Fos	2.698*	.303	.000
		Fos	Arc	-1.232*	.303	.001
			BDNF	1.536*	.303	.000
			Creb	.954*	.303	.038
			Egr1	.233	.340	1.000
	BDNF	Erk1	Erk1	-.665	.303	.611
		Erk2	Erk2	-2.698*	.303	.000
		Arc	BDNF	3.260*	.303	.000
		Creb	Creb	2.691*	.303	.000
1hr	Arc	Egr1	Egr1	1.300*	.303	.001
		Erk1	Erk1	.960*	.303	.036
		Erk2	Erk2	-1.087*	.303	.008
		Fos	Fos	1.333*	.303	.000
		BDNF	Arc	-3.260*	.303	.000
	BDNF	Creb	Creb	-.569	.303	1.000
		Egr1	Egr1	-1.960*	.303	.000
		Erk1	Erk1	-2.300*	.303	.000
		Erk2	Erk2	-4.347*	.303	.000
		Fos	Fos	-1.927*	.303	.000
2hr	Creb	Creb	Arc	-2.691*	.303	.000
		BDNF	BDNF	.569	.303	1.000
		Egr1	Egr1	-1.391*	.303	.000
		Erk1	Erk1	-1.731*	.303	.000
		Erk2	Erk2	-3.778*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	10 min	Fos	Fos	-1.357*	.303	.000
			Egr1	-1.300*	.303	.001
		BDNF	BDNF	1.960*	.303	.000
			Creb	1.391*	.303	.000
		Erk1	Erk1	-.340	.303	1.000
			Erk2	-2.387*	.303	.000
	1 hr	Fos	Fos	.034	.303	1.000
			Erk1	-.960*	.303	.036
		BDNF	BDNF	2.300*	.303	.000
			Creb	1.731*	.303	.000
		Egr1	Egr1	.340	.303	1.000
			Erk2	-2.047*	.303	.000
Prefrontal cortex	10 min	Fos	Fos	.374	.303	1.000
			Erk2	1.087*	.303	.008
		BDNF	BDNF	4.347*	.303	.000
			Creb	3.778*	.303	.000
		Egr1	Egr1	2.387*	.303	.000
			Erk1	2.047*	.303	.000
	1 hr	Fos	Fos	2.421*	.303	.000
			Fos	-1.333*	.303	.000
		BDNF	BDNF	1.927*	.303	.000
			Creb	1.357*	.303	.000
		Egr1	Egr1	-.034	.303	1.000
			Erk1	-.374	.303	1.000
		Erk2	Erk2	-2.421*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
30min	Arc	BDNF	Arc	3.170*	.303	.000
			Creb	2.879*	.303	.000
			Egr1	1.030*	.303	.016
			Erk1	.759	.303	.269
			Erk2	-1.192*	.303	.002
	BDNF	Arc	Fos	1.617*	.303	.000
			Creb	-3.170*	.303	.000
			Egr1	-.291	.303	1.000
			Erk1	-2.140*	.303	.000
			Erk2	-2.411*	.303	.000
Creb	Arc	BDNF	Fos	-4.362*	.303	.000
			Creb	-1.554*	.303	.000
			Egr1	-2.879*	.303	.000
			Erk1	.291	.303	1.000
			Erk2	-1.849*	.303	.000
	Egr1	Arc	Erk1	-2.120*	.303	.000
			Erk2	-4.071*	.303	.000
			Fos	-1.263*	.303	.001
			BDNF	-1.030*	.303	.016
			Creb	2.140*	.303	.000
Erk1	Arc	BDNF	Egr1	1.849*	.303	.000
			Erk1	-.271	.303	1.000
			Erk2	-2.222*	.303	.000
			Fos	.586	.303	1.000
			Creb	-.759	.303	.269

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1hr	Erk2	BDNF	2.411*	.303	.000
			Creb	2.120*	.303	.000
			Egr1	.271	.303	1.000
			Erk2	-1.951*	.303	.000
			Fos	.857	.303	.106
		Fos	Arc	1.192*	.303	.002
			BDNF	4.362*	.303	.000
			Creb	4.071*	.303	.000
			Egr1	2.222*	.303	.000
			Erk1	1.951*	.303	.000
Hippocampus	1hr	Fos	Fos	2.808*	.303	.000
			Arc	-1.617*	.303	.000
			BDNF	1.554*	.303	.000
			Creb	1.263*	.303	.001
			Egr1	-.586	.303	1.000
		60min	Erk1	-.857	.303	.106
			Erk2	-2.808*	.303	.000
			Arc	2.607*	.303	.000
			BDNF	2.528*	.303	.000
			Egr1	.921	.303	.055
Prefrontal Cortex	1hr	Arc	Erk1	.682	.303	.528
			Erk2	-1.280*	.303	.001
			Fos	1.631*	.303	.000
	60min	BDNF	Arc	-2.607*	.303	.000
			Creb	-.079	.303	1.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Cerb	0	Egr1		-1.686*	.303	.000
		Erk1		-1.925*	.303	.000
		Erk2		-3.887*	.303	.000
		Fos		-.977*	.303	.030
		Arc		-2.528*	.303	.000
	1	BDNF		.079	.303	1.000
		Egr1		-1.607*	.303	.000
		Erk1		-1.846*	.303	.000
		Erk2		-3.808*	.303	.000
		Fos		-.898	.303	.070
Egr1	0	Arc		-.921	.303	.055
		BDNF		1.686*	.303	.000
		Creb		1.607*	.303	.000
		Erk1		-.239	.303	1.000
		Erk2		-2.201*	.303	.000
	1	Fos		.710	.303	.419
		Arc		-.682	.303	.528
		BDNF		1.925*	.303	.000
		Creb		1.846*	.303	.000
		Egr1		.239	.303	1.000
Erk1	0	Erk2		-1.962*	.303	.000
		Fos		.948*	.303	.041
		Arc		1.280*	.303	.001
	1	BDNF		3.887*	.303	.000
		Creb		3.808*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
CER	pre	Arc	Egr1	2.201*	.303	.000
			Erk1	1.962*	.303	.000
			Fos	2.910*	.303	.000
			Fos	-1.631*	.303	.000
			BDNF	.977*	.303	.030
			Creb	.898	.303	.070
			Egr1	-.710	.303	.419
			Erk1	-.948*	.303	.041
			Erk2	-2.910*	.303	.000
			BDNF	-.712	.371	1.000
CER	post	Arc	Creb	-.935	.371	.260
			Egr1	.036	.371	1.000
			Erk1	-1.994*	.371	.000
			Erk2	-3.127*	.371	.000
			Fos	.896	.371	.345
			BDNF	.712	.371	1.000
			Creb	-.223	.371	1.000
			Egr1	.748	.371	.941
			Erk1	-1.282*	.371	.013
			Erk2	-2.415*	.371	.000
CER	delay	Arc	Fos	1.608*	.371	.000
			Creb	.935	.371	.260
			BDNF	.223	.371	1.000
			Egr1	.971	.371	.198
CER	delay	BDNF	Erk1	-1.060	.371	.097

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.	
Amygdala	0 min	Erk2		-2.193*	.371	.000	
			Fos	1.831*	.371	.000	
		Egr1	Arc	-.036	.371	1.000	
			BDNF	-.748	.371	.941	
			Creb	-.971	.371	.198	
	1 hr	Erk1		-2.030*	.371	.000	
			Erk2	-3.163*	.371	.000	
		Erk1	Fos	.860	.371	.446	
			Arc	1.994*	.371	.000	
			BDNF	1.282*	.371	.013	
Prefrontal Cortex	0 min	Creb	Creb	1.060	.371	.097	
			Egr1	2.030*	.371	.000	
			Erk2	-1.133	.371	.052	
			Fos	2.891*	.371	.000	
		Erk2	Arc	3.127*	.371	.000	
	1 hr		BDNF	2.415*	.371	.000	
			Creb	2.193*	.371	.000	
			Egr1	3.163*	.371	.000	
			Erk1	1.133	.371	.052	
	Fos	Fos	4.024*	.371	.000		
Hippocampus	0 min	Fos	Arc	-.896	.371	.345	
			BDNF	-1.608*	.371	.000	
			Creb	-1.831*	.371	.000	
			Egr1	-.860	.371	.446	
			Erk1	-2.891*	.371	.000	

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
0min	Arc	Erk2	BDNF	-4.024*	.371	.000
			Creb	-1.032*	.303	.016
			Egr1	-.292	.303	1.000
			Erk1	-2.126*	.303	.000
			Fos	-3.182*	.303	.000
	BDNF	Arc	Erk2	-2.130*	.303	.000
			Creb	1.032*	.303	.016
			Egr1	.012	.303	1.000
			Erk1	.739	.303	.322
			Fos	-1.095*	.303	.008
Creb	Arc	Erk2	BDNF	-2.150*	.303	.000
			Creb	1.020*	.303	.018
			Egr1	-.012	.303	1.000
			Erk1	.728	.303	.358
			Fos	-1.106*	.303	.007
	Egr1	Arc	BDNF	-2.162*	.303	.000
			Creb	-.110*	.303	.006
			Egr1	.292	.303	1.000
			Erk1	-.739	.303	.322
			Fos	-.728	.303	.358
		Erk1	BDNF	-1.834*	.303	.000
			Creb	-1.889*	.303	.000
			Fos	-1.837*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	1hr	Erk1	Arc	2.126*	.303	.000
			BDNF	1.095*	.303	.008
			Creb	1.106*	.303	.007
			Egr1	1.834*	.303	.000
			Erk2	-1.055*	.303	.012
		Erk2	Fos	-.003	.303	1.000
			Arc	3.182*	.303	.000
			BDNF	2.150*	.303	.000
			Creb	2.162*	.303	.000
			Egr1	2.889*	.303	.000
Prefrontal Cortex	1hr	Erk1	Erk1	1.055*	.303	.012
			Fos	1.052*	.303	.013
		Fos	Arc	2.130*	.303	.000
			BDNF	1.098*	.303	.007
			Creb	1.110*	.303	.006
		15min	Egr1	1.837*	.303	.000
			Erk1	.003	.303	1.000
			Erk2	-1.052*	.303	.013
			Arc	-1.159*	.303	.003
			BDNF	-1.040*	.303	.015
Hippocampus	1hr	Arc	Egr1	-.373	.303	1.000
			Erk1	-2.042*	.303	.000
			Erk2	-3.116*	.303	.000
			Fos	-2.216*	.303	.000
			BDNF	Arc	1.159*	.303

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Creb		Creb		.119	.303	1.000
		Egr1		.786	.303	.210
		Erk1		-.883	.303	.082
		Erk2		-1.957*	.303	.000
		Fos		-1.057*	.303	.012
		Creb	Arc	1.040*	.303	.015
		BDNF		-.119	.303	1.000
		Egr1		.667	.303	.600
		Erk1		-1.002*	.303	.023
		Erk2		-2.076*	.303	.000
Egr1		Fos		-1.176*	.303	.003
		Egr1	Arc	.373	.303	1.000
		BDNF		-.786	.303	.210
		Creb		-.667	.303	.600
		Erk1		-1.669*	.303	.000
		Erk2		-2.743*	.303	.000
		Fos		-1.843*	.303	.000
		Erk1	Arc	2.042*	.303	.000
		BDNF		.883	.303	.082
		Creb		1.002*	.303	.023
Erk2		Egr1		1.669*	.303	.000
		Erk2		-1.074*	.303	.010
		Fos		-.174	.303	1.000
		Erk2	Arc	3.116*	.303	.000
		BDNF		1.957*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.	
Amygdala	0min	Fos	Creb	2.076*	.303	.000	
			Egr1	2.743*	.303	.000	
			Erk1	1.074*	.303	.010	
			Fos	.900	.303	.068	
		Arc	BDNF	2.216*	.303	.000	
	30min	Fos	Creb	1.057*	.303	.012	
			Egr1	1.176*	.303	.003	
			Erk1	1.843*	.303	.000	
			Erk2	.174	.303	1.000	
		Arc	BDNF	-.900	.303	.068	
Hippocampus	0min	Arc	BDNF	-1.096*	.303	.007	
			Creb	-.908	.303	.063	
			Egr1	-.200	.303	1.000	
			Erk1	-1.940*	.303	.000	
		BDNF	Erk2	-3.095*	.303	.000	
	30min		Fos	-1.757*	.303	.000	
			Arc	1.096*	.303	.007	
			Creb	.188	.303	1.000	
			Egr1	.896	.303	.071	
			Erk1	-.844	.303	.120	
Prefrontal Cortex	0min	Arc	Erk2	-1.999*	.303	.000	
			Fos	-.661	.303	.633	
		Creb	Arc	.908	.303	.063	
	30min	BDNF	BDNF	-.188	.303	1.000	
			Egr1	.708	.303	.424	

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
		Erk1		-1.032*	.303	.016
		Erk2		-2.187*	.303	.000
		Fos		-.849	.303	.115
	Egr1	Arc		.200	.303	1.000
		BDNF		-.896	.303	.071
		Creb		-.708	.303	.424
		Erk1		-1.740*	.303	.000
		Erk2		-2.895*	.303	.000
		Fos		-1.557*	.303	.000
	Erk1	Arc		1.940*	.303	.000
		BDNF		.844	.303	.120
		Creb		1.032*	.303	.016
		Egr1		1.740*	.303	.000
		Erk2		-1.155*	.303	.004
		Fos		.184	.303	1.000
	Erk2	Arc		3.095*	.303	.000
		BDNF		1.999*	.303	.000
		Creb		2.187*	.303	.000
		Egr1		2.895*	.303	.000
		Erk1		1.155*	.303	.004
		Fos		1.339*	.303	.000
	Fos	Arc		1.757*	.303	.000
		BDNF		.661	.303	.633
		Creb		.849	.303	.115
		Egr1		1.557*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
60min	Arc	BDNF	Erk1	-.184	.303	1.000
			Erk2	-1.339*	.303	.000
			Creb	-.857	.303	.105
			Egr1	-.723	.303	.374
			Erk1	.125	.303	1.000
	BDNF	Arc	Erk2	-1.863*	.303	.000
			Fos	-2.921*	.303	.000
			Creb	-.176*	.303	.003
			Egr1	.857	.303	.105
			Erk1	.135	.303	1.000
Creb	Arc	BDNF	Egr1	.982*	.303	.028
			Erk1	-1.006*	.303	.022
			Erk2	-2.064*	.303	.000
			Fos	-.319	.303	1.000
			Creb	.723	.303	.374
	BDNF	Arc	BDNF	-.135	.303	1.000
			Egr1	.847	.303	.117
			Erk1	-1.141*	.303	.004
			Erk2	-2.199*	.303	.000
			Fos	-.453	.303	1.000
Egr1	Arc	BDNF	Egr1	-.125	.303	1.000
			BDNF	-.982*	.303	.028
			Creb	-.847	.303	.117
			Erk1	-1.988*	.303	.000
			Erk2	-3.046*	.303	.000

Region	Delay	(A) Gene	(B) Gene	(A-B) Mean Difference	Std. Error	Sig.
Amygdala	0 min	Fos		-1.301*	.303	.001
			Erk1	1.863*	.303	.000
			BDNF	1.006*	.303	.022
			Creb	1.141*	.303	.004
			Egr1	1.988*	.303	.000
	1 hr	Erk2		-1.058*	.303	.012
			Fos	.687	.303	.507
			Arc	2.921*	.303	.000
			BDNF	2.064*	.303	.000
			Creb	2.199*	.303	.000
Prefrontal cortex	0 min	Egr1		3.046*	.303	.000
			Erk1	1.058*	.303	.012
			Fos	1.745*	.303	.000
			Arc	1.176*	.303	.003
			BDNF	.319	.303	1.000
	1 hr	Creb		.453	.303	1.000
			Egr1	1.301*	.303	.001
			Erk1	-.687	.303	.507
			Erk2	-1.745*	.303	.000

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